

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/10391 A2

(51) International Patent Classification⁷: **C12N 15/12**,
C07K 14/705, 16/28, C12Q 1/68, G01N 33/68

(21) International Application Number: PCT/US01/24190

(22) International Filing Date: 31 July 2001 (31.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/221,925 31 July 2000 (31.07.2000) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

Published:

— without international search report and to be republished
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



WO 02/10391 A2

(54) Title: 18610, A NOVEL HUMAN TRANSIENT RECEPTOR AND USES THEREOF

(57) Abstract: The invention provides isolated nucleic acids molecules, designated TR-1 nucleic acid molecules, which encode novel transient receptor potential channel molecules. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing TR-1 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a TR-1 gene has been introduced or disrupted. The invention still further provides isolated TR-1 polypeptides, fusion polypeptides, antigenic peptides and anti-TR-1 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

18610, A NOVEL HUMAN TRANSIENT RECEPTOR AND USES THEREOF**Related Applications**

This application claims the benefit of prior-filed provisional patent application
5 Serial No. 60/221,925, filed July 31, 2000, entitled "18610, A NOVEL HUMAN
TRANSIENT RECEPTOR AND USES THEREOF." The entire contents of the above-
referenced application are incorporated herein by this reference.

Background of the Invention

10 Calcium signaling has been implicated in the regulation of a variety of cellular
responses, such as growth and differentiation. There are two general methods by which
intracellular concentrations of calcium ions may be increased: calcium ions may be
freed from intracellular stores, transported by specific membrane channels in the storage
organelle, or calcium ions may be brought into the cell from the extracellular milieu
15 through the use of specific channels in the cellular membrane. In the situation in which
the intracellular stores of calcium have been depleted, a specific type of calcium
channel, termed a 'capacitative calcium channel' or a 'store-operated calcium channel'
(SOC), is activated in the plasma membrane to import calcium ions from the
extracellular environment to the cytosol (see Putney and McKay (1999) *BioEssays*
20 21:38-46). Calcium may also enter the cell via receptor-stimulated cation channels (see
Hofmann *et al.* (2000) *J. Mol. Med.* 78:14-25).

There is no single electrophysiological profile characteristic of the calcium
channel family; rather, a wide array of single channel conductances, cation selectivity,
and current properties have been observed for different channels. Further, in several
25 instances it has been demonstrated that homo- or hetero-polymerization of the channel
molecule may occur, further changing the channel properties from those of the single
molecule. In general, though, these channels function similarly, in that they are calcium
ion-permeable cation channels which become activated after agonist binding to a G
protein-coupled receptor.

30 Members of the capacitative calcium channel family include the calcium release-
activated calcium current (CRAC) (Hoth and Penner (1992) *Nature* 355: 353-355),
calcium release-activated non-selective cation current (CRANC) (Krause *et al.* (1996) *J.*
Biol. Chem. 271: 32523-32528), and the transient receptor potential (TRP) proteins

TRP1, TRP2, TRP4, and TRP5. Depletion of intracellular calcium stores activate these channels by a mechanism which is yet undefined, but which has been demonstrated to involve a diffusible factor using studies in which calcium stores were artificially depleted (*e.g.*, by the introduction of chelators into the cell, by activating phospholipase C_γ, or by inhibiting those enzymes responsible for pumping calcium ions into the stores or those enzymes responsible for maintaining resting intracellular calcium ion concentrations) (Putney, J.W. (1986) *Cell Calcium* 7:1-12; Putney, J.W. (1990) *Cell Calcium* 11:611-624).

Recently, it has been elucidated that three TRP family members, TRP3, TRP6, and a mouse homologue, TRP7, form a sub-family of receptors that are activated in a calcium store-depletion independent manner. TRP3 and TRP6 are activated by diacylglycerols in a membrane delimited manner (Hofmann *et al.* (1999) *Nature* 397:259-263). Similarly, murine TRP7 is activated via diacylglycerol stimulation by G_q protein coupled receptors (Okada *et al.* (1999) *J. Biol. Chem.* 274:27359-27370).

The TRP channel family is one of the best characterized calcium channel protein families. These channels include transient receptor potential proteins and homologues thereof (to date, seven TRP homologues and splice variants have been identified in a variety of organisms), the vanilloid receptor subtype I (also known as the capsaicin receptor); the stretch-inhibitable non-selective cation channel (SIC); the olfactory, mechanosensitive channel; the insulin-like growth factor I-regulated calcium channel; the vitamin D-responsive apical, epithelial calcium channel (ECaC); and melastatin, and the polycystic kidney disease protein family (see, *e.g.*, Montell and Rubin (1989) *Neuron* 2:1313-1323; Caterina *et al.* (1997) *Nature* 389: 816-824; Suzuki *et al.* (1999) *J. Biol. Chem.* 274: 6330-6335; Kiselyov *et al.* (1998) *Nature* 396: 478-482; Hoenderop *et al.* (1999) *J. Biol. Chem.* 274: 8375-8378; and Chen *et al.* (1999) *Nature* 401(6751): 383-386). Each of these molecules is 700 or more amino acids in length, and shares certain conserved structural features. Predominant among these structural features are six transmembrane domains, with an additional hydrophobic loop present between the fifth and sixth transmembrane domains. It is believed that this loop is integral to the activity of the pore of the channel formed upon membrane insertion (Hardie and Minke (1993) *Trends Neurosci* 16: 371-376). Although found in disparate tissues and organisms, members of the TRP channel protein family all serve to transduce signals by means of calcium entry into cells, particularly pain signals (see, *e.g.*, McClesky and

Gold (1999) *Annu. Rev. Physiol.* 61: 835-856; Harteneck, C. (2000) *Trends Neurosci.* 23(4):159), light signals (Hardie and Minke, *supra*), or olfactory signals (Colbert *et al.* (1997) *J. Neurosci* 17(21): 8259-8269). Thus, this family of molecules may play important roles in sensory signal transduction in general.

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Summary of the Invention

The present invention is based, at least in part, on the discovery of novel transient receptor potential family members, referred to herein as “transient receptor-1” or “TR-1” nucleic acid and polypeptide molecules. The TR-1 nucleic acid and polypeptide molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes, *e.g.*, membrane excitability; neurite outgrowth and synaptogenesis; signal transduction; cell proliferation, growth, differentiation, and migration; and nociception. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding TR-1 polypeptides or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of TR-1-encoding nucleic acids.

In one embodiment, the invention features an isolated nucleic acid molecule that includes the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3. In another embodiment, the invention features an isolated nucleic acid molecule that encodes a polypeptide including the amino acid sequence set forth in SEQ ID NO:2. In another embodiment, the invention features an isolated nucleic acid molecule that includes the nucleotide sequence contained in the plasmid deposited with ATCC® as Accession Number ____.

In still other embodiments, the invention features isolated nucleic acid molecules including nucleotide sequences that are substantially identical (*e.g.*, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical) to the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3. The invention further features isolated nucleic acid molecules including at least 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 3914, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4640, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000,

6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7300 or more contiguous nucleotides of the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3. In another embodiment, the invention features isolated nucleic acid molecules which encode a polypeptide including an amino acid sequence that is

5 substantially identical (*e.g.*, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical) to the amino acid sequence set forth as SEQ ID NO:2. The present invention also features nucleic acid molecules which encode allelic variants of the polypeptide having the amino acid sequence set forth as SEQ ID NO:2. In addition to isolated nucleic acid molecules

10 encoding full-length polypeptides, the present invention also features nucleic acid molecules which encode fragments, for example, biologically active or antigenic fragments, of the full-length polypeptides of the present invention (*e.g.*, fragments including at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650,

15 675, 700, 725, 750, 775, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1301, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, or 1750 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2). In still other embodiments, the invention features nucleic acid molecules that are complementary to, antisense to, or hybridize under stringent conditions to the isolated nucleic acid molecules described

20 herein.

In another aspect, the invention provides vectors including the isolated nucleic acid molecules described herein (*e.g.*, TR-1-encoding nucleic acid molecules). Such vectors can optionally include nucleotide sequences encoding heterologous polypeptides. Also featured are host cells including such vectors (*e.g.*, host cells

25 including vectors suitable for producing TR-1 nucleic acid molecules and polypeptides).

In another aspect, the invention features isolated TR-1 polypeptides and/or biologically active or antigenic fragments thereof. Exemplary embodiments feature a polypeptide including the amino acid sequence set forth as SEQ ID NO:2, a polypeptide including an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%,

30 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth as SEQ ID NO:2, a polypeptide encoded by a nucleic acid molecule including a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical

to the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3. Also featured are fragments of the full-length polypeptides described herein (*e.g.*, fragments including at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1301, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, or 1750 contiguous amino acid residues of the sequence set forth as SEQ ID NO:2) as well as allelic variants of the polypeptide having the amino acid sequence set forth as SEQ ID NO:2. In another preferred embodiment, the nucleic acid molecule includes a fragment of at least 3,914 or 4,640 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or a complement thereof.

The TR-1 polypeptides and/or biologically active or antigenic fragments thereof, are useful, for example, as reagents or targets in assays applicable to treatment and/or diagnosis of TR-1 mediated or related disorders. In one embodiment, a TR-1 polypeptide or fragment thereof, has a TR-1 activity. In another embodiment, a TR-1 polypeptide or fragment thereof, has a transmembrane domain, a pore domain, a transient receptor domain, and optionally, has a TR-1 activity. In a related aspect, the invention features antibodies (*e.g.*, antibodies which specifically bind to any one of the polypeptides described herein) as well as fusion polypeptides including all or a fragment of a polypeptide described herein.

The present invention further features methods for detecting TR-1 polypeptides and/or TR-1 nucleic acid molecules, such methods featuring, for example, a probe, primer or antibody described herein. Also featured are kits *e.g.*, kits for the detection of TR-1 polypeptides and/or TR-1 nucleic acid molecules. In a related aspect, the invention features methods for identifying compounds which bind to and/or modulate the activity of a TR-1 polypeptide or TR-1 nucleic acid molecule described herein. Further featured are methods for modulating a TR-1 activity.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1A-F depicts the cDNA sequence and predicted amino acid sequence of human TR-1. The nucleotide sequence corresponds to nucleic acids 1 to 7334 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 1885 of SEQ ID NO: 2. The coding region without the 3' untranslated region of the human TR-1 gene is shown in SEQ ID NO: 3.

Figure 2 depicts a structural, hydrophobicity, and antigenicity analysis of the human TR-1 polypeptide which resulted in the identification of six "transmembrane domains" and a "pore domain," in the human TR-1 polypeptide.

Figure 3 is a graphic depiction of the relative levels of human TR-1 (clone Fbh18610) mRNA expression in a human tissue panel, as determined using Taqman™ analysis (1=lymph node, 2=spleen tissue, 3=thymus tissue, 4=brain tissue, 5=lung tissue, 6=skeletal muscle, 7=fetal liver tissue, 8=tonsil tissue, 9=colon tissue, 10=heart tissue, 11=liver tissue, 12=liver tissue, 13=liver fibrosis, 14=liver fibrosis, 15=liver fibrosis, 16=liver fibrosis, 17=liver fibrosis, 18=liver fibrosis, 19=liver fibrosis, 20=K-562 (chronic myelogenous leukemia) cells, 21=HL-60 (promyelocytic leukemia) cells, 22=Hep-3B (hepatocellular cells), normal, 23=Hep-3B (hepatocellular) hypoxia cells, 24=CD341⁺ cells, 25=CD341⁺ cells, 26=CD341⁺ cells, 27=Th1 cells, 6 hr., 28=Th1 cells, 48 hr., 29=Th1 cells, 48 hr., 30=Th2 cells, 6 hr., 31=Th2 cells, 48 hr., 32=Th2 cells, 48 hr., 33=CD3 cells, 34=CD4 cells, 35=CD8 cells, 36=B-cell, resting, 37=B-cell, activated, 38=PMBC resting, 39=PMBC activated, 40=CD14 cells, 41=granulocytes, 42=human embryonic kidney cells, 43=Jurkat cells, 44=CD34⁺, 45=leukocytes, 46=CD34⁺ cells).

Detailed Description of the Invention

The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as "transient receptor" or "TR-1" nucleic acid and polypeptide molecules, which are novel members of the transient receptor potential channel family. Transient receptor potential channel family members are ion channels, *e.g.*, calcium channels. These novel molecules are capable of, for example, modulating an ion-channel mediated activity (*e.g.*, a calcium channel-mediated activity) in a cell, *e.g.*, a neuronal, muscle (*e.g.*, cardiac muscle), or liver cell.

As used herein, an "ion channel" includes a protein or polypeptide which is involved in receiving, conducting, and transmitting signals in an electrically excitable cell, *e.g.*, a neuronal or muscle cell. Ion channels include calcium channels, potassium channels, and sodium channels. As used herein, a "calcium channel" includes a protein or polypeptide which is involved in receiving, conducting, and transmitting calcium ion-based signals in an electrically excitable cell. Calcium channels are calcium ion selective, and can determine membrane excitability (the ability of, for example, a neuronal cell to respond to a stimulus and to convert it into a sensory impulse). Calcium channels can also influence the resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation. Calcium channels are typically expressed in electrically excitable cells, *e.g.*, neuronal cells, and may form heteromultimeric structures (*e.g.*, composed of more than one type of subunit). Calcium channels may also be found in non-excitable cells (*e.g.*, adipose cells or liver cells), where they may play a role in, *e.g.*, signal transduction. Calcium channels are described in, for example, Davila *et al.* (1999) *Annals New York Academy of Sciences* 868:102-17 and McEnery, M.W. *et al.* (1998) *J. Bioenergetics and Biomembranes* 30(4): 409-418, the contents of which are incorporated herein by reference. As the TR-1 molecules of the present invention are calcium channels modulating ion channel mediated activities (*e.g.*, calcium channel mediated activities), they may be useful for developing novel diagnostic and therapeutic agents for ion channel associated disorders (*e.g.*, calcium channel associated disorders).

As used herein, an "ion channel associated disorder" includes a disorder, disease or condition which is characterized by a misregulation of an ion channel mediated activity. For example, a "calcium channel associated disorder" includes a disorder, disease or condition which is characterized by a misregulation of a calcium channel mediated activity. Ion channel associated disorders, *e.g.*, calcium channel associated disorders, include CNS disorders, such as cognitive and neurodegenerative disorders, examples of which include, but are not limited to, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, senile dementia, Huntington's disease, Gilles de la Tourette's syndrome, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, Jakob-Creutzfeldt disease, or AIDS related dementia; autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric

disorders, such as depression, schizophrenia, schizoaffective disorder, korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss, attention deficit disorder, psychoactive substance use disorders, anxiety, phobias, panic disorder, as well as bipolar affective disorder, *e.g.*, severe bipolar affective (mood) disorder (BP-1), and bipolar affective neurological disorders, *e.g.*, migraine and obesity. Further CNS-related disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical manual of Mental Disorders (DSM), the most current version of which is incorporated herein by reference in its entirety.

10 Ion channel associated disorders, *e.g.*, calcium channel disorders, also include pain disorders. Pain disorders include those that affect pain signaling mechanisms. As used herein, the term "pain signaling mechanisms" includes the cellular mechanisms involved in the development and regulation of pain, *e.g.*, pain elicited by noxious chemical, mechanical, or thermal stimuli, in a subject, *e.g.*, a mammal such as a human.

15 In mammals, the initial detection of noxious chemical, mechanical, or thermal stimuli, a process referred to as "nociception", occurs predominantly at the peripheral terminals of specialized, small diameter sensory neurons. These sensory neurons transmit the information to the central nervous system, evoking a perception of pain or discomfort and initiating appropriate protective reflexes. The TR-1 molecules of the present

20 invention may be present on these sensory neurons and, thus, may be involved in detecting these noxious chemical, mechanical, or thermal stimuli and transducing this information into membrane depolarization events. Thus, the TR-1 molecules by participating in pain signaling mechanisms, may modulate pain elicitation and act as targets for developing novel diagnostic targets and therapeutic agents to control pain.

25 Ion channel associated disorders, *e.g.*, calcium channel disorders, also include cellular proliferation, growth, differentiation, or migration disorders. Cellular proliferation, growth, differentiation, or migration disorders include those disorders that affect cell proliferation, growth, differentiation, or migration processes. As used herein, a "cellular proliferation, growth, differentiation, or migration process" is a process by

30 which a cell increases in number, size or content, by which a cell develops a specialized set of characteristics which differ from that of other cells, or by which a cell moves closer to or further from a particular location or stimulus. The TR-1 molecules of the present invention are involved in signal transduction mechanisms, which are known to

be involved in cellular growth, differentiation, and migration processes. Thus, the TR-1 molecules may modulate cellular growth, differentiation, or migration, and may play a role in disorders characterized by aberrantly regulated growth, differentiation, or migration. Such disorders include cancer, *e.g.*, carcinoma, sarcoma, or leukemia; tumor angiogenesis and metastasis; skeletal dysplasia; neuronal deficiencies resulting from impaired neural induction and patterning; hepatic disorders; cardiovascular disorders; and hematopoietic and/or myeloproliferative disorders.

As used herein, an "ion channel mediated activity" includes an activity which involves an ion channel, *e.g.*, an ion channel in a neuronal cell, a muscular cell, or a liver cell, associated with receiving, conducting, and transmitting signals, in, for example, the nervous system. Ion channel mediated activities (*e.g.*, calcium channel mediated activities) include release of neurotransmitters or second messenger molecules (*e.g.*, dopamine or norepinephrine), from cells, *e.g.*, neuronal cells; modulation of resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation; participation in signal transduction pathways, and modulation of processes such as integration of sub-threshold synaptic responses and the conductance of back-propagating action potentials in, for example, neuronal cells (*e.g.*, changes in those action potentials resulting in a morphological or differentiative response in the cell).

The term "family" when referring to the polypeptide and nucleic acid molecules of the invention is intended to mean two or more polypeptides or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first polypeptide of human origin, as well as other, distinct polypeptides of human origin or alternatively, can contain homologues of non-human origin, *e.g.*, monkey polypeptides. Members of a family may also have common functional characteristics.

For example, the family of TR-1 polypeptides comprise at least one "transmembrane domain" and preferably six transmembrane domains. As used herein, the term "transmembrane domain" includes an amino acid sequence of about 10-30 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 10, 15, 20, 25, or 30 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in hydrophobic

residues, and typically have an alpha-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, alanines, valines, phenylalanines, prolines or methionines. Transmembrane domains are described in, for example, Zagotta W.N. *et al.*, (1996) *Annual Rev. Neurosci.* 19: 235-263, the contents of which are incorporated herein by reference. Amino acid residues 758-774, 856-876, 923-941, 957-974, 1000-1016, and 1071-1096 of the TR-1 polypeptide comprise transmembrane domains (see Figure 2). Accordingly, TR-1 polypeptides having at least 50-60% homology, preferably about 60-70%, more preferably about 70-80%, or about 80-90% homology with a transmembrane domain of human TR-1 are within the scope of the invention.

In another embodiment, a TR-1 molecule of the present invention is identified based on the presence of at least one pore domain between the fifth and sixth transmembrane domains. As used herein, the term "pore domain" includes an overall hydrophobic amino acid sequence which is located between two transmembrane domains of a calcium channel protein, preferably transmembrane domains 5 and 6, and which is believed to be a major determinant of ion selectivity and channel activity in calcium channels. Pore domains are described in, for example Vannier *et al.* (1998) *J. Biol. Chem.* 273: 8675-8679 and Phillips, A. M. *et al.* (1992) *Neuron* 8, 631-642, the contents of which are incorporated herein by reference. TR-1 molecules having at least one pore domain are within the scope of the invention. A pore domain is found in the human TR-1 sequence (SEQ ID NO:2) at about residues 1036-1055 (Figure 2).

In another embodiment, a TR-1 molecule of the present invention is identified based on the presence of at least one "transient receptor domain." As used herein, the term "transient receptor domain" includes a protein domain having an amino acid sequence of about 40-175 amino acid residues which serves to transport ions. Preferably, a transient receptor domain includes at least about 48 amino acid residues. To identify the presence of a transient receptor domain in a TR-1 protein, and make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein may be searched against a database of known protein domains (*e.g.*, the HMM database). The transient receptor domain (HMM) has been assigned the PFAM Accession PF02164 (<http://genome.wustl.edu/Pfam/html>). A search was performed against the HMM database resulting in the identification of three transient receptor

domains in the amino acid sequence of human TR-1 (SEQ ID NO:2) at about residues 699-747, 849-1016, and 1079-1137 of SEQ ID NO:2.

A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28:405-420 and a detailed description of HMMs can be found, for example, in Gribkov *et al.* (1990) *Meth. Enzymol.* 183:146-159; Gribkov *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.* (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.* (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference.

In a preferred embodiment, the TR-1 molecules of the invention include at least one transmembrane domain, preferably six transmembrane domains, at least one pore domain, and/or at least one transient receptor domain.

Isolated polypeptides of the present invention, preferably TR-1 polypeptides, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or are encoded by a nucleotide sequence sufficiently identical to SEQ ID NO:1 or 3. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homology or identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide sequences which share at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homology or identity and share a common functional activity are defined herein as sufficiently identical.

In a preferred embodiment, a TR-1 polypeptide includes at least one or more of the following domains: a transmembrane domain, and/or a pore domain, and/or a transient receptor domain, and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous or identical to the amino acid sequence of SEQ ID

NO:2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In yet another preferred embodiment, a TR-1 polypeptide includes at least one or more of the following domains: a transmembrane domain, and/or a pore domain, and/or a transient receptor domain, and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3. In another preferred embodiment, a TR-1 polypeptide includes at least one or more of the following domains: a transmembrane domain, and/or a pore domain, and/or a transient receptor domain, and has a TR-1 activity.

As used interchangeably herein, a "TR-1 activity", "biological activity of TR-1" or "functional activity of TR-1", refers to an activity exerted by a TR-1 polypeptide or nucleic acid molecule on a TR-1 responsive cell or tissue, or on a TR-1 polypeptide substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, a TR-1 activity is a direct activity, such as an association with a TR-1-target molecule. As used herein, a "substrate," "target molecule," or "binding partner" is a molecule with which a TR-1 polypeptide binds or interacts in nature, such that TR-1-mediated function is achieved. A TR-1 target molecule can be a non-TR-1 molecule or a TR-1 polypeptide or polypeptide of the present invention. In an exemplary embodiment, a TR-1 target molecule is a TR-1 ligand, *e.g.*, a calcium channel ligand such as calcium. Alternatively, a TR-1 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the TR-1 polypeptide with a TR-1 ligand. The biological activities of TR-1 are described herein. For example, the TR-1 polypeptides of the present invention can have one or more of the following activities:

- (1) modulate membrane excitability, (2) influence the resting potential of membranes, (3) modulate wave forms and frequencies of action potentials, (4) modulate thresholds of excitation, (5) modulate neurite outgrowth and synaptogenesis, (6) modulate signal transduction, (7) participate in nociception, and (8) bind and transport calcium ions.

The nucleotide sequence of the isolated human TR-1 cDNA and the predicted amino acid sequence of the human TR-1 polypeptide are shown in Figure 1A-F and in SEQ ID NOs:1 and 2, respectively. A plasmid containing the nucleotide sequence encoding human TR-1 was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and

assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35

5 U.S.C. §112.

The human TR-1 gene, which is approximately 7334 nucleotides in length, encodes a polypeptide which is approximately 1885 amino acid residues in length.

Various aspects of the invention are described in further detail in the following subsections:

10

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode TR-1 polypeptides or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify TR-1-encoding nucleic acid molecules (*e.g.*, TR-1 mRNA) and fragments for use as PCR primers for the
15 amplification or mutation of TR-1 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-
20 stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which
25 the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated TR-1 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of
30 nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material,

or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, as a hybridization probe, TR-1 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to TR-1 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

Accordingly, in another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:3 and nucleotides 1-257 and 5913-7334 of SEQ ID NO:1. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3.

In one embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1 or 3. This cDNA may comprise sequences encoding the human TR-1 protein (*e.g.*, the "coding region", from nucleotides 258-5912), as well as 5' untranslated sequence (nucleotides 1-257) and 3' untranslated

sequences (nucleotides 5913-7334) of SEQ ID NO:1. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (e.g., nucleotides 258-5912, corresponding to SEQ ID NO:3). Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention comprises SEQ ID NO:3 and nucleotides 1-257 of SEQ ID NO:1. In yet another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:3 and nucleotides 5913-7334 of SEQ ID NO:1. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3.

In still another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence shown in SEQ ID NO:1 or 3 (e.g., to the entire length of the nucleotide sequence), or to the nucleotide sequence (e.g., the entire length of the nucleotide sequence) of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences. In one embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least (or no greater than) 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600,

3700, 3800, 3900, 3914, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4640, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7300, or more nucleotides in length and hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a TR-1 polypeptide, *e.g.*, a biologically active portion of a TR-1 polypeptide. The nucleotide sequence determined from the cloning of the TR-1 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other TR-1 family members, as well as TR-1 homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The probe/primer (*e.g.*, oligonucleotide) typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, 75, 80, 85, 90, 95, or 100 or more consecutive nucleotides of a sense sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, of an anti-sense sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

Exemplary probes or primers are at least (or no greater than) 12 or 15, 20 or 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or more nucleotides in length and/or comprise consecutive nucleotides of an isolated nucleic acid molecule described herein. Probes based on the TR-1 nucleotide sequences can be used to detect (*e.g.*, specifically detect) transcripts or genomic sequences encoding the same or homologous polypeptides. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme

co-factor. In another embodiment a set of primers is provided, *e.g.*, primers suitable for use in a PCR, which can be used to amplify a selected region of a TR-1 sequence, *e.g.*, a domain, region, site or other sequence described herein. The primers should be at least 5, 10, or 50 base pairs in length and less than 100, or less than 200, base pairs in length.

- 5 Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a TR-1 polypeptide, such as by measuring a level of a TR-1-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting TR-1 mRNA levels or determining whether a genomic TR-1 gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a TR-1 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, which encodes a polypeptide having a TR-1 biological activity (the biological activities of the TR-1 polypeptides are described herein), expressing the encoded portion of the TR-1 polypeptide (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the TR-1 polypeptide. In an exemplary embodiment, the nucleic acid molecule is at least 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 3914, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4640, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7300 or more nucleotides in length and encodes a polypeptide having a TR-1 activity (as described herein).

- 25 The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. Such differences can be due to the degeneracy of the genetic code, thus resulting in a nucleic acid which encodes the same TR-1 polypeptides as those encoded by the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a polypeptide having an amino acid sequence which differs by at
- 30

least 1, but no greater than 5, 10, 20, 50, 100, or 200 amino acid residues from the amino acid sequence shown in SEQ ID NO:2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____. In yet another embodiment, the nucleic acid molecule encodes the amino acid sequence of human TR-1. If an alignment is needed for this comparison, the sequences should be aligned for maximum homology.

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologues (different locus), and orthologues (different organism) or can be non naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

Allelic variants result, for example, from DNA sequence polymorphisms within a population (*e.g.*, the human population) that lead to changes in the amino acid sequences of the TR-1 polypeptides. Such genetic polymorphism in the TR-1 genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a TR-1 polypeptide, preferably a mammalian TR-1 polypeptide, and can further include non-coding regulatory sequences, and introns.

Accordingly, in one embodiment, the invention features isolated nucleic acid molecules which encode a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the nucleic acid molecule hybridizes to a complement of a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:3, for example, under stringent hybridization conditions.

Allelic variants of human TR-1 include both functional and non-functional TR-1 polypeptides. Functional allelic variants are naturally occurring amino acid sequence variants of the human TR-1 polypeptide that maintain the ability to bind a TR-1 ligand or substrate and/or modulate membrane excitability or signal transduction. Functional

allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the polypeptide.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human TR-1 polypeptide that do not have the ability to form functional calcium channels or to modulate membrane excitability. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2, or a substitution, insertion or deletion in critical residues or critical regions.

The present invention further provides non-human and non-murine orthologues (*e.g.*, non-human and non-murine orthologues of the human TR-1 polypeptide). Orthologues of the human TR-1 polypeptides are polypeptides that are isolated from non-human organisms and possess the same TR-1 ligand binding and/or modulation of membrane excitation mechanisms of the human TR-1 polypeptide. Orthologues of the human TR-1 polypeptide can readily be identified as comprising an amino acid sequence that is substantially identical to SEQ ID NO:2.

Moreover, nucleic acid molecules encoding other TR-1 family members and, thus, which have a nucleotide sequence which differs from the TR-1 sequences of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ are intended to be within the scope of the invention. For example, another TR-1 cDNA can be identified based on the nucleotide sequence of human TR-1. Moreover, nucleic acid molecules encoding TR-1 polypeptides from different species, and which, thus, have a nucleotide sequence which differs from the TR-1 sequences of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ are intended to be within the scope of the invention. For example, a hamster TR-1 cDNA can be identified based on the nucleotide sequence of a human TR-1.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the TR-1 cDNAs of the invention can be isolated based on their homology to the TR-1 nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural allelic

variants and homologues of the TR-1 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the TR-1 gene.

Orthologues, homologues and allelic variants can be identified using methods known in the art (*e.g.*, by hybridization to an isolated nucleic acid molecule of the present invention, for example, under stringent hybridization conditions). In one embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In other embodiments, the nucleic acid molecule is at least 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 3914, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4640, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7300 or more nucleotides in length.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or

more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(^{\circ}\text{C}) = 2(\# \text{ of A + T bases}) + 4(\# \text{ of G + C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\% \text{G+C}) - (600/N)$, where N is the number of bases in the hybrid, and $[\text{Na}^+]$ is the concentration of sodium ions in the hybridization buffer ($[\text{Na}^+]$ for 1xSSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH₂PO₄, 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH₂PO₄, 1% SDS at 65°C, see *e.g.*, Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or alternatively 0.2X SSC, 1% SDS).

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 or 3 and corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural polypeptide).

In addition to naturally-occurring allelic variants of the TR-1 sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1 or 3, or the

nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby leading to changes in the amino acid sequence of the encoded TR-1 polypeptides, without altering the functional ability of the TR-1 polypeptides. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of TR-1 (*e.g.*, the sequence of SEQ ID NO:2) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the TR-1 polypeptides of the present invention, *e.g.*, those present in a transmembrane domain, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the TR-1 polypeptides of the present invention and other members of the TR-1 family are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding TR-1 polypeptides that contain changes in amino acid residues that are not essential for activity. Such TR-1 polypeptides differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide, wherein the polypeptide comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2 (*e.g.*, to the entire length of SEQ ID NO:2).

An isolated nucleic acid molecule encoding a TR-1 polypeptide identical to the polypeptide of SEQ ID NO:2, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded polypeptide. Mutations can be introduced into SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains
5 (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue
10 in a TR-1 polypeptide is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a TR-1 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for TR-1 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or 3, or the
15 nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, the encoded polypeptide can be expressed recombinantly and the activity of the polypeptide can be determined.

In a preferred embodiment, a mutant TR-1 polypeptide can be assayed for the ability to (1) modulate membrane excitability, (2) influence the resting potential of
20 membranes, (3) modulate wave forms and frequencies of action potentials, (4) modulate thresholds of excitation, (5) modulate neurite outgrowth and synaptogenesis, (6) modulate signal transduction, (7) participate in nociception, and (8) bind and transport calcium ions.

In addition to the nucleic acid molecules encoding TR-1 polypeptides described
25 above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. In an exemplary embodiment, the invention provides an isolated nucleic acid molecule which is antisense to a TR-1 nucleic acid molecule (*e.g.*, is antisense to the coding strand of a TR-1 nucleic acid molecule). An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid
30 encoding a polypeptide, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire TR-1 coding strand, or to only a portion thereof. In one

embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding TR-1. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of human TR-1 corresponds to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding TR-1. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding TR-1 disclosed herein (*e.g.*, SEQ ID NO:3), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of TR-1 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of TR-1 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of TR-1 mRNA (*e.g.*, between the -10 and +10 regions of the start site of a gene nucleotide sequence). An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-

methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N⁶-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

10 The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a TR-1 polypeptide to thereby inhibit expression of the polypeptide, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, 15 in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic 20 administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the 25 antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the 30 usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes
5 (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave TR-1 mRNA transcripts to thereby inhibit translation of TR-1 mRNA. A ribozyme having specificity for a TR-1-encoding nucleic acid can be designed based upon the nucleotide sequence of a TR-1 cDNA disclosed herein (*i.e.*, SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid
10 deposited with ATCC as Accession Number _____). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a TR-1-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, TR-1 mRNA can be used to select a catalytic RNA
15 having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, TR-1 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the TR-1 (*e.g.*, the TR-1 promoter and/or enhancers) to form triple helical structures that prevent transcription of the TR-1
20 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In yet another embodiment, the TR-1 nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to
25 improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate
30 backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide

synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs of TR-1 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for
5 sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of TR-1 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or
10 primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of TR-1 can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of
15 drug delivery known in the art. For example, PNA-DNA chimeras of TR-1 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of
20 appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and
25 modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be
30 synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; 5 PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization 10 triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

Alternatively, the expression characteristics of an endogenous TR-1 gene within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous TR-1 15 gene. For example, an endogenous TR-1 gene which is normally "transcriptionally silent", *i.e.*, a TR-1 gene which is normally not expressed, or is expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a transcriptionally silent, 20 endogenous TR-1 gene may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous TR-1 gene, using techniques, such as targeted homologous recombination, which are well known to 25 those of skill in the art, and described, *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

II. Isolated TR-1 Polypeptides and Anti-TR-1 Antibodies

One aspect of the invention pertains to isolated TR-1 or recombinant 30 polypeptides and polypeptides, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-TR-1 antibodies. In one embodiment, native TR-1 polypeptides can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In

another embodiment, TR-1 polypeptides are produced by recombinant DNA techniques. Alternative to recombinant expression, a TR-1 polypeptide or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the TR-1 polypeptide is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of TR-1 polypeptide in which the polypeptide is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of TR-1 polypeptide having less than about 30% (by dry weight) of non-TR-1 polypeptide (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-TR-1 polypeptide, still more preferably less than about 10% of non-TR-1 polypeptide, and most preferably less than about 5% non-TR-1 polypeptide. When the TR-1 polypeptide or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of TR-1 polypeptide in which the polypeptide is separated from chemical precursors or other chemicals which are involved in the synthesis of the polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of TR-1 polypeptide having less than about 30% (by dry weight) of chemical precursors or non-TR-1 chemicals, more preferably less than about 20% chemical precursors or non-TR-1 chemicals, still more preferably less than about 10% chemical precursors or non-TR-1 chemicals, and most preferably less than about 5% chemical precursors or non-TR-1 chemicals.

As used herein, a "biologically active portion" of a TR-1 polypeptide includes a fragment of a TR-1 polypeptide which participates in an interaction between a TR-1 molecule and a non-TR-1 molecule. Biologically active portions of a TR-1 polypeptide include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the TR-1 polypeptide, *e.g.*, the amino acid sequence

shown in SEQ ID NO:2, which include less amino acids than the full length TR-1 polypeptides, and exhibit at least one activity of a TR-1 polypeptide. Typically, biologically active portions comprise a domain or motif with at least one activity of the TR-1 polypeptide, *e.g.*, modulating membrane excitation mechanisms. A biologically active portion of a TR-1 polypeptide can be a polypeptide which is, for example, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1301, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, or more amino acids in length. Biologically active portions of a TR-1 polypeptide can be used as targets for developing agents which modulate a TR-1 mediated activity, *e.g.*, a membrane excitation mechanism.

In one embodiment, a biologically active portion of a TR-1 polypeptide comprises at least one transmembrane domain. It is to be understood that a preferred biologically active portion of a TR-1 polypeptide of the present invention comprises at least one or more of the following domains: a transmembrane domain, a pore domain, and/or a transient receptor domain. Moreover, other biologically active portions, in which other regions of the polypeptide are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native TR-1 polypeptide.

Another aspect of the invention features fragments of the polypeptide having the amino acid sequence of SEQ ID NO:2, for example, for use as immunogens. In one embodiment, a fragment comprises at least 5 amino acids (*e.g.*, contiguous or consecutive amino acids) of the amino acid sequence of SEQ ID NO:2, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____. In another embodiment, a fragment comprises at least 10, 15, 20, 25, 30, 35, 40, 45, 50 or more amino acids (*e.g.*, contiguous or consecutive amino acids) of the amino acid sequence of SEQ ID NO:2, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____.

In a preferred embodiment, a TR-1 polypeptide has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the TR-1 polypeptide is substantially identical to SEQ ID NO:2, and retains the functional activity of the polypeptide of SEQ ID NO:2, yet differs in amino acid sequence due to natural allelic variation or

mutagenesis, as described in detail in subsection I above. In another embodiment, the TR-1 polypeptide is a polypeptide which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2.

5 In another embodiment, the invention features a TR-1 polypeptide which is encoded by a nucleic acid molecule consisting of a nucleotide sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, or a complement thereof. This invention further features a TR-1 polypeptide
10 which is encoded by a nucleic acid molecule consisting of a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, or a complement thereof.

To determine the percent identity of two amino acid sequences or of two nucleic
15 acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more
20 preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the TR-1 amino acid sequence of SEQ ID NO:2 having 1885 amino acid residues, at least 622, preferably at least 654, more preferably at least 942, more preferably at least 1131, even more preferably at least 1319, and even more preferably at
25 least 1508 or 1696 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid
30 "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which
5 has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at
10 <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A preferred, non-limiting example of parameters to be used in conjunction with the GAP program include a Blosum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

15 In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or version 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

20 The nucleic acid and polypeptide sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the
25 NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to TR-1 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 100, wordlength = 3, and a Blosum62 matrix to obtain amino acid sequences homologous to TR-1 polypeptide molecules of the invention. To obtain gapped alignments for comparison purposes,
30 Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

The invention also provides TR-1 chimeric or fusion proteins. As used herein, a TR-1 "chimeric protein" or "fusion protein" comprises a TR-1 polypeptide operatively linked to a non-TR-1 polypeptide. A "TR-1 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to TR-1, whereas a "non-TR-1 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a polypeptide which is not substantially homologous to the TR-1 polypeptide, *e.g.*, a polypeptide which is different from the TR-1 polypeptide and which is derived from the same or a different organism. Within a TR-1 fusion protein the TR-1 polypeptide can correspond to all or a portion of a TR-1 polypeptide. In a preferred embodiment, a TR-1 fusion protein comprises at least one biologically active portion of a TR-1 polypeptide. In another preferred embodiment, a TR-1 fusion protein comprises at least two biologically active portions of a TR-1 polypeptide. Within the fusion protein, the term "operatively linked" is intended to indicate that the TR-1 polypeptide and the non-TR-1 polypeptide are fused in-frame to each other. The non-TR-1 polypeptide can be fused to the N-terminus or C-terminus of the TR-1 polypeptide.

For example, in one embodiment, the fusion protein is a GST-TR-1 fusion protein in which the TR-1 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant TR-1.

In another embodiment, the fusion protein is a TR-1 polypeptide containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of TR-1 can be increased through the use of a heterologous signal sequence.

The TR-1 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The TR-1 fusion proteins can be used to affect the bioavailability of a TR-1 substrate. Use of TR-1 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a TR-1 polypeptide; (ii) mis-regulation of the TR-1 gene; and (iii) aberrant post-translational modification of a TR-1 polypeptide.

Moreover, the TR-1-fusion proteins of the invention can be used as immunogens to produce anti-TR-1 antibodies in a subject, to purify TR-1 ligands and in screening assays to identify molecules which inhibit the interaction of TR-1 with a TR-1 substrate.

Preferably, a TR-1 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended
5 termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor
10 primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A TR-1-
15 encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the TR-1 polypeptide.

The present invention also pertains to variants of the TR-1 polypeptides which function as either TR-1 agonists (mimetics) or as TR-1 antagonists. Variants of the TR-1 polypeptides can be generated by mutagenesis, *e.g.*, discrete point mutation or
20 truncation of a TR-1 polypeptide. An agonist of the TR-1 polypeptides can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a TR-1 polypeptide. An antagonist of a TR-1 polypeptide can inhibit one or more of the activities of the naturally occurring form of the TR-1 polypeptide by, for example, competitively modulating a TR-1-mediated activity of a TR-1 polypeptide.
25 Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the polypeptide has fewer side effects in a subject relative to treatment with the naturally occurring form of the TR-1 polypeptide.

30 In one embodiment, variants of a TR-1 polypeptide which function as either TR-1 agonists (mimetics) or as TR-1 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a TR-1 polypeptide for TR-1 polypeptide agonist or antagonist activity. In one embodiment, a variegated

library of TR-1 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of TR-1 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential TR-1 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of TR-1 sequences therein. There are a variety of methods which can be used to produce libraries of potential TR-1 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential TR-1 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of a TR-1 polypeptide coding sequence can be used to generate a variegated population of TR-1 fragments for screening and subsequent selection of variants of a TR-1 polypeptide. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a TR-1 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the TR-1 polypeptide.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TR-1 polypeptides. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting

library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in

5 combination with the screening assays to identify TR-1 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated TR-1 library. For example, a library of expression vectors can be transfected into a cell
10 line, *e.g.*, an endothelial cell line, which ordinarily responds to TR-1 in a particular TR-1 substrate-dependent manner. The transfected cells are then contacted with TR-1 and the effect of expression of the mutant on signaling by the TR-1 substrate can be detected, *e.g.*, by monitoring intracellular calcium, IP3, or diacylglycerol concentration, phosphorylation profile of intracellular proteins, or the activity of a TR-1-regulated
15 transcription factor. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the TR-1 substrate, and the individual clones further characterized.

An isolated TR-1 polypeptide, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind TR-1 using standard techniques for
20 polyclonal and monoclonal antibody preparation. A full-length TR-1 polypeptide can be used or, alternatively, the invention provides antigenic peptide fragments of TR-1 for use as immunogens. The antigenic peptide of TR-1 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of TR-1 such that an antibody raised against the peptide forms a specific
25 immune complex with TR-1. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of TR-1 that
30 are located on the surface of the polypeptide, *e.g.*, hydrophilic regions, as well as regions with high antigenicity (see, for example, Figure 2).

A TR-1 immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed TR-1 polypeptide or a chemically synthesized TR-1 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic TR-1 preparation induces a polyclonal anti-TR-1 antibody response.

Accordingly, another aspect of the invention pertains to anti-TR-1 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as TR-1. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind TR-1. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of TR-1. A monoclonal antibody composition thus typically displays a single binding affinity for a particular TR-1 polypeptide with which it immunoreacts.

Polyclonal anti-TR-1 antibodies can be prepared as described above by immunizing a suitable subject with a TR-1 immunogen. The anti-TR-1 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized TR-1. If desired, the antibody molecules directed against TR-1 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-TR-1 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the

more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a TR-1 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds TR-1.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-TR-1 monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind TR-1, *e.g.*, using a standard ELISA assay.

- Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-TR-1 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with TR-1 to thereby isolate immunoglobulin library members that bind TR-1.
- 5 Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S.
- 10 Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT
- 15 International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad.*
- 20 *Sci. USA* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* *Nature* (1990) 348:552-554.
- Additionally, recombinant anti-TR-1 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions,
- 25 which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496;
- 30 Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.*

(1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; 5 Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoevan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-TR-1 antibody (*e.g.*, monoclonal antibody) can be used to isolate TR-1 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-TR-1 antibody can facilitate the purification of natural TR-1 from cells and of 10 recombinantly produced TR-1 expressed in host cells. Moreover, an anti-TR-1 antibody can be used to detect TR-1 polypeptide (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the TR-1 polypeptide. Anti-TR-1 antibodies can be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a 15 given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or 20 acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include 25 luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, for example recombinant 30 expression vectors, containing a nucleic acid containing a TR-1 nucleic acid molecule or vectors containing a nucleic acid molecule which encodes a TR-1 polypeptide (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of

vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*,
5 bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to
10 herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective
15 retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory
20 sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a
25 host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which
30 direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the

host cell to be transformed, the level of expression of polypeptide desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., TR-1 polypeptides, mutant forms of TR-1 polypeptides, fusion proteins, and the like).

Accordingly, an exemplary embodiment provides a method for producing a polypeptide, preferably a TR-1 polypeptide, by culturing in a suitable medium a host cell of the invention (e.g., a mammalian host cell such as a non-human mammalian cell) containing a recombinant expression vector, such that the polypeptide is produced.

The recombinant expression vectors of the invention can be designed for expression of TR-1 polypeptides in prokaryotic or eukaryotic cells. For example, TR-1 polypeptides can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in TR-1 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for TR-1 polypeptides, for example. In a preferred embodiment, a TR-1 fusion protein expressed in a retroviral expression vector of the present invention can be
5 utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene*
10 *Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral
15 polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the
20 recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118).
25 Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the TR-1 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-
30 943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, TR-1 polypeptides can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 5 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsch, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY,* 15 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 25 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to TR-1 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA.

The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a TR-1 nucleic acid molecule of the invention is introduced, *e.g.*, a TR-1 nucleic acid molecule within a vector (*e.g.*, a recombinant expression vector) or a TR-1 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a TR-1 polypeptide can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including

calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor*
5 *Laboratory Press, Cold Spring Harbor, NY, 1989*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is
10 generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a TR-1 polypeptide or can be introduced on a separate vector. Cells stably transfected with the introduced
15 nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a TR-1 polypeptide. Accordingly, the invention further provides methods for producing a TR-1 polypeptide using the host
20 cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a TR-1 polypeptide has been introduced) in a suitable medium such that a TR-1 polypeptide is produced. In another embodiment, the method further comprises isolating a TR-1 polypeptide from the medium or the host cell.

25 The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which TR-1-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous TR-1 sequences have been introduced into their genome or
30 homologous recombinant animals in which endogenous TR-1 sequences have been altered. Such animals are useful for studying the function and/or activity of a TR-1 and for identifying and/or evaluating modulators of TR-1 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a

rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous TR-1 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing a TR-1-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The TR-1 cDNA sequence of SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human TR-1 gene, such as a mouse or rat TR-1 gene, can be used as a transgene. Alternatively, a TR-1 gene homologue, such as another TR-1 family member, can be isolated based on hybridization to the TR-1 cDNA sequences of SEQ ID NO:1 or 3, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____ (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a TR-1 transgene to direct expression of a TR-1 polypeptide to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a TR-1 transgene in its genome and/or expression of TR-1 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed

additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a TR-1 polypeptide can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains
5 at least a portion of a TR-1 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the TR-1 gene. The TR-1 gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:3), but more preferably, is a non-human homologue of a human TR-1 gene (*e.g.*, a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1). For example, a mouse
10 TR-1 gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous TR-1 gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous TR-1 gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out"
15 vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous TR-1 gene is mutated or otherwise altered but still encodes functional polypeptide (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous TR-1 polypeptide). In the homologous recombination nucleic acid molecule, the altered
20 portion of the TR-1 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the TR-1 gene to allow for homologous recombination to occur between the exogenous TR-1 gene carried by the homologous recombination nucleic acid molecule and an endogenous TR-1 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking TR-1 nucleic acid sequence is of sufficient length for successful homologous
25 recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by
30 electroporation) and cells in which the introduced TR-1 gene has homologously recombined with the endogenous TR-1 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then be injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and*

Embryonic Stem Cells: A Practical Approach, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

IV. Pharmaceutical Compositions

The TR-1 nucleic acid molecules, fragments of TR-1 polypeptides, and anti-TR-1 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, polypeptide, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy

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syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid
5 polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol,
10 ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

15 Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a fragment of a TR-1 polypeptide or an anti-TR-1 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains
20 a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

25 Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is
30 applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as

microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject

to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to

the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a polypeptide or antibody can include a single treatment or, preferably, can include a series of treatments.

5 In a preferred example, a subject is treated with antibody or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody or polypeptide used for treatment may
10 increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino
15 acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000
20 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for
25 example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule
30 per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram.

It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin

such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"),
5 interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug
10 Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future
15 Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982).
Alternatively, an antibody can be conjugated to a second antibody to form an antibody
20 heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-
25 3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene
30 delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

5 The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, a TR-1 polypeptide of the invention has one or more
10 of the following activities: (1) modulates membrane excitability, (2) influences the resting potential of membranes, (3) modulates wave forms and frequencies of action potentials, (4) modulates thresholds of excitation, (5) modulates neurite outgrowth and synaptogenesis, (6) modulates signal transduction, (7) participates in nociception, and (8) bind and transport calcium ions.

15 The isolated nucleic acid molecules of the invention can be used, for example, to express TR-1 polypeptide (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect TR-1 mRNA (*e.g.*, in a biological sample) or a genetic alteration in a TR-1 gene, and to modulate TR-1 activity, as described further below. The TR-1 polypeptides can be used to treat disorders characterized by
20 insufficient or excessive production of a TR-1 substrate or production of TR-1 inhibitors. In addition, the TR-1 polypeptides can be used to screen for naturally occurring TR-1 substrates, to screen for drugs or compounds which modulate TR-1 activity, as well as to treat disorders characterized by insufficient or excessive production of TR-1 polypeptide or production of TR-1 polypeptide forms which have
25 decreased, aberrant or unwanted activity compared to TR-1 wild type polypeptide (*e.g.*, CNS disorders (such as neurodegenerative disorders), pain disorders, or cellular growth, differentiation, or migration disorders). Moreover, the anti-TR-1 antibodies of the invention can be used to detect and isolate TR-1 polypeptides, to regulate the bioavailability of TR-1 polypeptides, and modulate TR-1 activity.

A. Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to TR-1 polypeptides, 5 have a stimulatory or inhibitory effect on, for example, TR-1 expression or TR-1 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of TR-1 substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a TR-1 polypeptide or polypeptide or biologically 10 active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a TR-1 polypeptide or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially 15 addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. 20 (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in 25 Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP 30 '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a TR-1 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate TR-1 activity is determined. Determining the ability of the test compound to modulate TR-1 activity can be accomplished by monitoring, for example, intracellular calcium, IP3, or diacylglycerol concentration, phosphorylation profile of intracellular proteins, or the activity of a TR-1-regulated transcription factor. The cell, for example, can be of mammalian origin, *e.g.*, a neuronal cell, or a liver cell.

The ability of the test compound to modulate TR-1 binding to a substrate or to bind to TR-1 can also be determined. Determining the ability of the test compound to modulate TR-1 binding to a substrate can be accomplished, for example, by coupling the TR-1 substrate with a radioisotope or enzymatic label such that binding of the TR-1 substrate to TR-1 can be determined by detecting the labeled TR-1 substrate in a complex. Alternatively, TR-1 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate TR-1 binding to a TR-1 substrate in a complex. Determining the ability of the test compound to bind TR-1 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to TR-1 can be determined by detecting the labeled TR-1 compound in a complex. For example, compounds (*e.g.*, TR-1 substrates) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, a TR-1 substrate) to interact with TR-1 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with TR-1 without the labeling of either the compound or the TR-1. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and TR-1.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a TR-1 target molecule (*e.g.*, a TR-1 substrate) with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the TR-1 target molecule. Determining the ability of the test compound to
5 modulate the activity of a TR-1 target molecule can be accomplished, for example, by determining the ability of the TR-1 polypeptide to bind to or interact with the TR-1 target molecule.

Determining the ability of the TR-1 polypeptide, or a biologically active fragment thereof, to bind to or interact with a TR-1 target molecule can be accomplished
10 by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the TR-1 polypeptide to bind to or interact with a TR-1 target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.*, intracellular Ca^{2+} ,
15 diacylglycerol, IP_3 , and the like), detecting catalytic/enzymatic activity of the target using an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response.

In yet another embodiment, an assay of the present invention is a cell-free assay
20 in which a TR-1 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the TR-1 polypeptide or biologically active portion thereof is determined. Preferred biologically active portions of the TR-1 polypeptides to be used in assays of the present invention include fragments which participate in interactions with non-TR-1 molecules, *e.g.*, fragments with high
25 surface probability scores (see, for example, Figure 2). Binding of the test compound to the TR-1 polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the TR-1 polypeptide or biologically active portion thereof with a known compound which binds TR-1 to form an assay mixture, contacting the assay mixture with a test compound, and determining
30 the ability of the test compound to interact with a TR-1 polypeptide, wherein determining the ability of the test compound to interact with a TR-1 polypeptide comprises determining the ability of the test compound to preferentially bind to TR-1 or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which a TR-1 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the TR-1 polypeptide or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a TR-1 polypeptide can be accomplished, for example, by determining the ability of the TR-1 polypeptide to bind to a TR-1 target molecule by one of the methods described above for determining direct binding. Determining the ability of the TR-1 polypeptide to bind to a TR-1 target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of a TR-1 polypeptide can be accomplished by determining the ability of the TR-1 polypeptide to further modulate the activity of a downstream effector of a TR-1 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a TR-1 polypeptide or biologically active portion thereof with a known compound which binds the TR-1 polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the TR-1 polypeptide, wherein determining the ability of the test compound to interact with the TR-1 polypeptide comprises determining the ability of the TR-1 polypeptide to preferentially bind to or modulate the activity of a TR-1 target molecule.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either TR-1 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a TR-1 polypeptide, or interaction of a TR-1 polypeptide with a target

molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to
5 be bound to a matrix. For example, glutathione-S-transferase/ TR-1 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized micrometer plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or TR-1 polypeptide, and the
10 mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or micrometer plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the
15 matrix, and the level of TR-1 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either a TR-1 polypeptide or a TR-1 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated TR-1 polypeptide or target molecules can be prepared from biotin-NHS (N-
20 hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with TR-1 polypeptide or target molecules but which do not interfere with binding of the TR-1 polypeptide to its target molecule can be derivatized to the wells of the plate, and unbound target or TR-1
25 polypeptide trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the TR-1 polypeptide or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the TR-1 polypeptide or target molecule.

30 In another embodiment, modulators of TR-1 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of TR-1 mRNA or polypeptide in the cell is determined. The level of expression of TR-1 mRNA or polypeptide in the presence of the candidate compound is compared to the

level of expression of TR-1 mRNA or polypeptide in the absence of the candidate compound. The candidate compound can then be identified as a modulator of TR-1 expression based on this comparison. For example, when expression of TR-1 mRNA or polypeptide is greater (statistically significantly greater) in the presence of the candidate
5 compound than in its absence, the candidate compound is identified as a stimulator of TR-1 mRNA or polypeptide expression. Alternatively, when expression of TR-1 mRNA or polypeptide is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of TR-1 mRNA or polypeptide expression. The level of TR-1 mRNA or
10 polypeptide expression in the cells can be determined by methods described herein for detecting TR-1 mRNA or polypeptide.

In yet another aspect of the invention, the TR-1 polypeptides can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.*
15 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with TR-1 ("TR-1-binding proteins" or "TR-1-bp") and are involved in TR-1 activity. Such TR-1-binding proteins are also likely to be involved in the propagation of signals by the TR-1 polypeptides or TR-1 targets as, for example,
20 downstream elements of a TR-1-mediated signaling pathway. Alternatively, such TR-1-binding proteins are likely to be TR-1 inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a
25 TR-1 polypeptide is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a TR-1-dependent
30 complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell

colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the TR-1 polypeptide.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a TR-1 polypeptide can be confirmed *in vivo*, e.g., in an animal such as an animal model for cellular transformation and/or tumorigenesis or an animal model for a neurodegenerative disorder, such as Alzheimer's disease. Animal based models for studying tumorigenesis *in vivo* are well known in the art (reviewed in Animal Models of Cancer Predisposition Syndromes, Hiai, H and Hino, O (eds.) 1999, *Progress in Experimental Tumor Research*, Vol. 35; Clarke AR *Carcinogenesis* (2000) 21:435-41) and include, for example, carcinogen-induced tumors (Rithidech, K *et al. Mutat Res* (1999) 428:33-39; Miller, ML *et al. Environ Mol Mutagen* (2000) 35:319-327), injection and/or transplantation of tumor cells into an animal, as well as animals bearing mutations in growth regulatory genes, for example, oncogenes (e.g., ras) (Arbeit, JM *et al. Am J Pathol* (1993) 142:1187-1197; Sinn, E *et al. Cell* (1987) 49:465-475; Thorgeirsson, SS *et al. Toxicol Lett* (2000) 112-113:553-555) and tumor suppressor genes (e.g., p53) (Vooijs, M *et al. Oncogene* (1999) 18:5293-5303; Clark AR *Cancer Metast Rev* (1995) 14:125-148; Kumar, TR *et al. J Intern Med* (1995) 238:233-238; Donehower, LA *et al.* (1992) *Nature* 356:215-221). Furthermore, experimental model systems are available for the study of, for example, ovarian cancer (Hamilton, TC *et al. Semin Oncol* (1984) 11:285-298; Rahman, NA *et al. Mol Cell Endocrinol* (1998) 145:167-174; Beamer, WG *et al. Toxicol Pathol* (1998) 26:704-710), gastric cancer (Thompson, J *et al. Int J Cancer* (2000) 86:863-869; Fodde, R *et al. Cytogenet Cell Genet* (1999) 86:105-111), breast cancer (Li, M *et al. Oncogene* (2000) 19:1010-1019; Green, JE *et al. Oncogene* (2000) 19:1020-1027), melanoma (Satyamoorthy, K *et al. Cancer Metast Rev* (1999) 18:401-405), and prostate cancer (Shirai, T *et al. Mutat Res* (2000) 462:219-226; Bostwick, DG *et al. Prostate* (2000) 43:286-294).

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a TR-1 modulating agent, an antisense TR-1 nucleic acid molecule, a TR-1-specific antibody, or a TR-1-binding partner) can be used

in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the TR-1 nucleotide sequences, described herein, can be used to map the location of the TR-1 genes on a chromosome. The mapping of the TR-1 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, TR-1 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the TR-1 nucleotide sequences. Computer analysis of the TR-1 sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the TR-1 sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they

lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse
5 chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a
10 particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the TR-1 nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a TR-1 sequence to its chromosome include *in situ* hybridization (described
15 in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in
20 one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence
25 as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques
30 (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to

noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the TR-1 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The TR-1 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the TR-1 nucleotide sequences

described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The TR-1 nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from TR-1 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of TR-1 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified

sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the TR-1 nucleotide sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1 having a length of at least 20 bases, preferably at least 30 bases.

The TR-1 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such TR-1 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, TR-1 primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining TR-1 polypeptide and/or nucleic acid expression as well as TR-1 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant or unwanted TR-1 expression or activity. The

invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with TR-1 polypeptide, nucleic acid expression or activity. For example, mutations in a TR-1 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to
5 thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with TR-1 polypeptide, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of TR-1 in clinical trials.

These and other agents are described in further detail in the following sections.

10

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of TR-1 polypeptide or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of
15 detecting TR-1 polypeptide or nucleic acid (*e.g.*, mRNA, or genomic DNA) that encodes TR-1 polypeptide such that the presence of TR-1 polypeptide or nucleic acid is detected in the biological sample. In another aspect, the present invention provides a method for detecting the presence of TR-1 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of TR-1 activity such
20 that the presence of TR-1 activity is detected in the biological sample. A preferred agent for detecting TR-1 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to TR-1 mRNA or genomic DNA. The nucleic acid probe can be, for example, the TR-1 nucleic acid set forth in SEQ ID NO:1 or 3, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, such as
25 an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to TR-1 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting TR-1 polypeptide is an antibody capable of
30 binding to TR-1 polypeptide, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by

coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect TR-1 mRNA, polypeptide, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of TR-1 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of TR-1 polypeptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of TR-1 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of TR-1 polypeptide include introducing into a subject a labeled anti-TR-1 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

The present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding a TR-1 polypeptide; (ii) aberrant expression of a gene encoding a TR-1 polypeptide; (iii) mis-regulation of the gene; and (iii) aberrant post-translational modification of a TR-1 polypeptide, wherein a wild-type form of the gene encodes a polypeptide with a TR-1 activity. "Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes, but is not limited to, expression at non-wild type levels (*e.g.*, over or under expression); a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed (*e.g.*, increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage); a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-translational modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the

effect of an environmental stimulus or extracellular stimulus on expression of the gene (*e.g.*, a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus).

In one embodiment, the biological sample contains protein molecules from the
5 test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control
biological sample from a control subject, contacting the control sample with a
10 compound or agent capable of detecting TR-1 polypeptide, mRNA, or genomic DNA, such that the presence of TR-1 polypeptide, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of TR-1 polypeptide, mRNA or genomic DNA in the control sample with the presence of TR-1 polypeptide, mRNA or genomic DNA in the test sample.

15 The invention also encompasses kits for detecting the presence of TR-1 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting TR-1 polypeptide or mRNA in a biological sample; means for determining the amount of TR-1 in the sample; and means for comparing the amount of TR-1 in the sample with a standard. The compound or agent can be packaged in a
20 suitable container. The kit can further comprise instructions for using the kit to detect TR-1 polypeptide or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify
25 subjects having or at risk of developing a disease or disorder associated with aberrant or unwanted TR-1 expression or activity. As used herein, the term "aberrant" includes a TR-1 expression or activity which deviates from the wild type TR-1 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type
30 developmental pattern of expression or the subcellular pattern of expression. For example, aberrant TR-1 expression or activity is intended to include the cases in which a mutation in the TR-1 gene causes the TR-1 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional TR-1

polypeptide or a polypeptide which does not function in a wild-type fashion, *e.g.*, a polypeptide which does not interact with a TR-1 substrate, *e.g.*, a non-calcium channel subunit or ligand, or one which interacts with a non-TR-1 substrate, *e.g.* a non-calcium channel subunit or ligand. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response, such as cellular proliferation. For example, the term unwanted includes a TR-1 expression or activity which is undesirable in a subject.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in TR-1 polypeptide activity or nucleic acid expression, such as a CNS disorder (*e.g.*, a neurodegenerative disorder, a pain disorder, or a cellular proliferation, growth, differentiation, or migration disorder). Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in TR-1 polypeptide activity or nucleic acid expression, such as a CNS disorder, a pain disorder, or a cellular proliferation, growth, differentiation, or migration disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant or unwanted TR-1 expression or activity in which a test sample is obtained from a subject and TR-1 polypeptide or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of TR-1 polypeptide or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted TR-1 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted TR-1 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for an ion channel associated disorder, *e.g.*, a CNS disorder, a pain disorder, or a cellular growth, differentiation, or migration disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant or unwanted TR-

1 expression or activity in which a test sample is obtained and TR-1 polypeptide or
nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of TR-1
polypeptide or nucleic acid expression or activity is diagnostic for a subject that can be
administered the agent to treat a disorder associated with aberrant or unwanted TR-1
5 expression or activity).

The methods of the invention can also be used to detect genetic alterations in a
TR-1 gene, thereby determining if a subject with the altered gene is at risk for a disorder
characterized by misregulation in TR-1 polypeptide activity or nucleic acid expression,
such as an ion channel associated disorder, *e.g.*, a CNS disorder, a pain disorder, or a
10 cellular growth, differentiation, or migration disorder. In preferred embodiments, the
methods include detecting, in a sample of cells from the subject, the presence or absence
of a genetic alteration characterized by at least one of an alteration affecting the integrity
of a gene encoding a TR-1 -polypeptide, or the mis-expression of the TR-1 gene. For
example, such genetic alterations can be detected by ascertaining the existence of at
15 least one of 1) a deletion of one or more nucleotides from a TR-1 gene; 2) an addition of
one or more nucleotides to a TR-1 gene; 3) a substitution of one or more nucleotides of a
TR-1 gene, 4) a chromosomal rearrangement of a TR-1 gene; 5) an alteration in the level
of a messenger RNA transcript of a TR-1 gene, 6) aberrant modification of a TR-1 gene,
such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild
20 type splicing pattern of a messenger RNA transcript of a TR-1 gene, 8) a non-wild type
level of a TR-1-polypeptide, 9) allelic loss of a TR-1 gene, and 10) inappropriate post-
translational modification of a TR-1-polypeptide. As described herein, there are a large
number of assays known in the art which can be used for detecting alterations in a TR-1
gene. A preferred biological sample is a tissue or serum sample isolated by
25 conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a
probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos.
4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a
ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080;
30 and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which
can be particularly useful for detecting point mutations in the TR-1-gene (see Abravaya
et al. (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of
collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA

or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a TR-1 gene under conditions such that hybridization and amplification of the TR-1-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the

5 amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional
10 amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such
15 molecules are present in very low numbers.

In an alternative embodiment, mutations in a TR-1 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel
20 electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

25 In other embodiments, genetic mutations in TR-1 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in TR-1 can be identified in two dimensional
30 arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the

identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other
5 complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the TR-1 gene and detect mutations by comparing the sequence of the sample TR-1 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques
10 developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen *et al.*
15 (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the TR-1 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the
20 art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type TR-1 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample
25 strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then
30 separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in TR-1 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a TR-1 sequence, *e.g.*, a wild-type TR-1 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in TR-1 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control TR-1 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is

used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective
5 primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different
10 mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention.

Oligonucleotides used as primers for specific amplification may carry the mutation of
15 interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based
20 detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence
25 of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a TR-1
30 gene.

Furthermore, any cell type or tissue in which TR-1 is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

5 Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a TR-1 polypeptide (*e.g.*, the modulation of membrane excitability) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase TR-1 gene expression, polypeptide levels, or upregulate TR-1 activity, can be monitored in clinical
10 trials of subjects exhibiting decreased TR-1 gene expression, polypeptide levels, or downregulated TR-1 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease TR-1 gene expression, polypeptide levels, or downregulate TR-1 activity, can be monitored in clinical trials of subjects exhibiting increased TR-1 gene expression, polypeptide levels, or upregulated TR-1 activity. In such clinical trials,
15 the expression or activity of a TR-1 gene, and preferably, other genes that have been implicated in, for example, a TR-1-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including TR-1, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule)
20 which modulates TR-1 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on TR-1-associated disorders (*e.g.*, disorders characterized by deregulated signaling or membrane excitation), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of TR-1 and other genes implicated in the TR-1-associated disorder,
25 respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of polypeptide produced, by one of the methods as described herein, or by measuring the levels of activity of TR-1 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response
30 of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a TR-1 polypeptide, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the TR-1 polypeptide, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the TR-1 polypeptide, mRNA, or genomic DNA in the pre-administration sample with the TR-1 polypeptide, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of TR-1 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of TR-1 to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, TR-1 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

D. Methods of Treatment:

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted TR-1 expression or activity, *e.g.*, an ion channel associated disorder, such as a CNS disorder, a pain disorder, or a cellular proliferation, growth, differentiation, or migration disorder. "Treatment", as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving or affecting the disease or disorder, the symptoms of disease or disorder or the predisposition toward a disease or disorder. A therapeutic

agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the TR-1 molecules of the present invention or TR-1 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted TR-1 expression or activity, by administering to the subject a TR-1 or an agent which modulates TR-1 expression or at least one TR-1 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted TR-1 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the TR-1 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of TR-1 aberrancy, for example, a TR-1, TR-1 agonist or TR-1 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating TR-1 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell capable
5 of expressing TR-1 with an agent that modulates one or more of the activities of TR-1 polypeptide activity associated with the cell, such that TR-1 activity in the cell is modulated. An agent that modulates TR-1 polypeptide activity can be an agent as described herein, such as a nucleic acid or a polypeptide, a naturally-occurring target molecule of a TR-1 polypeptide (*e.g.*, a TR-1 substrate), a TR-1 antibody, a TR-1
10 agonist or antagonist, a peptidomimetic of a TR-1 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more TR-1 activities. Examples of such stimulatory agents include active TR-1 polypeptide and a nucleic acid molecule encoding TR-1 that has been introduced into the cell. In another embodiment, the agent inhibits one or more TR-1 activities. Examples of such inhibitory agents
15 include antisense TR-1 nucleic acid molecules, anti-TR-1 antibodies, and TR-1 inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or
20 activity of a TR-1 polypeptide or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) TR-1 expression or activity. In another embodiment, the method involves administering a TR-1 polypeptide or nucleic acid molecule as therapy to
25 compensate for reduced, aberrant, or unwanted TR-1 expression or activity.

Stimulation of TR-1 activity is desirable in situations in which TR-1 is abnormally downregulated and/or in which increased TR-1 activity is likely to have a beneficial effect. Likewise, inhibition of TR-1 activity is desirable in situations in which TR-1 is abnormally upregulated and/or in which decreased TR-1 activity is likely to
30 have a beneficial effect.

3. Pharmacogenomics

The TR-1 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on TR-1 activity (e.g., TR-1 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) TR-1-associated disorders (e.g., proliferative disorders) associated with aberrant or unwanted TR-1 activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a TR-1 molecule or TR-1 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a TR-1 molecule or TR-1 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution

genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (*e.g.*, a TR-1 polypeptide of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-

formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to
5 identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a TR-1 molecule or TR-1 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics
10 approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a TR-1 molecule or TR-1 modulator, such as a modulator identified by one of the exemplary
15 screening assays described herein.

4. Use of TR-1 Molecules as Surrogate Markers

The TR-1 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition
20 of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the TR-1 molecules of the invention may be detected, and may be correlated with one or more biological states *in vivo*. For example, the TR-1 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or
25 for conditions leading up to disease states. As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (*e.g.*, with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of
30 treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (*e.g.*, early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is

reached (e.g., an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate
5 markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The TR-1 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a "pharmacodynamic marker" is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a
10 pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or
15 transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug *in vivo*.
20 Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., a TR-1 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more
25 easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-TR-1 antibodies may be employed in an immune-based detection system for a TR-1 polypeptide marker, or TR-1-specific radiolabeled probes may be used to detect a TR-1 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the
30 range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

The TR-1 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, *e.g.*, McLeod *et al.* (1999) *Eur. J. Cancer* 35(12): 1650-1652). The
5 presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the
10 presence or quantity of RNA, or polypeptide (*e.g.*, TR-1 polypeptide or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in TR-1 DNA may correlate TR-1 drug response. The use of pharmacogenomic markers therefore permits
15 the application of the most appropriate treatment for each subject without having to administer the therapy.

VI. Electronic Apparatus Readable Media and Arrays

Electronic apparatus readable media comprising TR-1 sequence information is
20 also provided. As used herein, "TR-1 sequence information" refers to any nucleotide and/or amino acid sequence information particular to the TR-1 molecules of the present invention, including but not limited to full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequences, and the like.
25 Moreover, information "related to" said TR-1 sequence information includes detection of the presence or absence of a sequence (*e.g.*, detection of expression of a sequence, fragment, polymorphism, etc.), determination of the level of a sequence (*e.g.*, detection of a level of expression, for example, a quantitative detection), detection of a reactivity to a sequence (*e.g.*, detection of protein expression and/or levels, for example, using a
30 sequence-specific antibody), and the like. As used herein, "electronic apparatus readable media" refers to any suitable medium for storing, holding or containing data or information that can be read and accessed directly by an electronic apparatus. Such media can include, but are not limited to: magnetic storage media, such as floppy discs,

hard disc storage medium, and magnetic tape; optical storage media such as compact disc; electronic storage media such as RAM, ROM, EPROM, EEPROM and the like; general hard disks and hybrids of these categories such as magnetic/optical storage media. The medium is adapted or configured for having recorded thereon TR-1
5 sequence information of the present invention.

As used herein, the term "electronic apparatus" is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the present invention include stand-alone computing apparatus; networks, including a local
10 area network (LAN), a wide area network (WAN) Internet, Intranet, and Extranet; electronic appliances such as a personal digital assistants (PDAs), cellular phone, pager and the like; and local and distributed processing systems.

As used herein, "recorded" refers to a process for storing or encoding information on the electronic apparatus readable medium. Those skilled in the art can
15 readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising the TR-1 sequence information.

A variety of software programs and formats can be used to store the sequence information on the electronic apparatus readable medium. For example, the sequence information can be represented in a word processing text file, formatted in
20 commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like, as well as in other forms. Any number of data processor structuring formats (*e.g.*, text file or database) may be employed in order to obtain or create a medium having recorded thereon the TR-1 sequence information.

By providing TR-1 sequence information in readable form, one can routinely
25 access the sequence information for a variety of purposes. For example, one skilled in the art can use the sequence information in readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the
30 invention which match a particular target sequence or target motif.

The present invention therefore provides a medium for holding instructions for performing a method for determining whether a subject has a TR-1- associated disease or disorder or a pre-disposition to a TR-1-associated disease or disorder, wherein the

method comprises the steps of determining TR-1 sequence information associated with the subject and based on the TR-1 sequence information, determining whether the subject has a TR-1-associated disease or disorder or a pre-disposition to a TR-1-associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a TR-1-associated disease or disorder or a pre-disposition to a disease associated with a TR-1 wherein the method comprises the steps of determining TR-1 sequence information associated with the subject, and based on the TR-1 sequence information, determining whether the subject has a TR-1 -associated disease or disorder or a pre-disposition to a TR-1-associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. The method may further comprise the step of receiving phenotypic information associated with the subject and/or acquiring from a network phenotypic information associated with the subject.

The present invention also provides in a network, a method for determining whether a subject has a TR-1-associated disease or disorder or a pre-disposition to a TR-1 associated disease or disorder associated with TR-1, said method comprising the steps of receiving TR-1 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to TR-1 and/or a TR-1-associated disease or disorder, and based on one or more of the phenotypic information, the TR-1 information (*e.g.*, sequence information and/or information related thereto), and the acquired information, determining whether the subject has a TR-1-associated disease or disorder or a pre-disposition to a TR-1-associated disease or disorder (*e.g.*, a CNS disorder, pain disorder, or a cellular proliferation, growth, differentiation, or migration disorder). The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a business method for determining whether a subject has a TR-1-associated disease or disorder or a pre-disposition to a TR-1-associated disease or disorder, said method comprising the steps of receiving information related to TR-1 (*e.g.*, sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring

information from the network related to TR-1 and/or related to a TR-1-associated disease or disorder, and based on one or more of the phenotypic information, the TR-1 information, and the acquired information, determining whether the subject has a TR-1-associated disease or disorder or a pre-disposition to a TR-1-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The invention also includes an array comprising a TR-1 sequence of the present invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 7600 genes can be simultaneously assayed for expression, one of which can be TR-1. This allows a profile to be developed showing a battery of genes specifically expressed in one or more tissues.

In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue expression *per se* and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development of a TR-1-associated disease or

disorder, progression of TR-1-associated disease or disorder, and processes, such a cellular transformation associated with the TR-1-associated disease or disorder.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (*e.g.*, ascertaining the effect of TR-1 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (*e.g.*, including TR-1) that could serve as a molecular target for diagnosis or therapeutic intervention.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are incorporated herein by reference.

EXAMPLES

EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF HUMAN

TR-1 cDNA

In this example, the identification and characterization of the gene encoding human TR-1 (clone Fbh18610) is described.

Isolation of the Human TR-1 cDNA

The invention is based, at least in part, on the discovery of a human gene encoding a novel polypeptide, referred to herein as human TR-1. The entire sequence of the human clone Fbh18610 was determined and found to contain an open reading frame termed human "TR-1." The nucleotide sequence of the human TR-1 gene is set forth in Figures 1A-F and in the Sequence Listing as SEQ ID NO:1. The amino acid sequence of the human TR-1 expression product is set forth in Figures 1A-F and in the Sequence Listing as SEQ ID NO:2. The TR-1 polypeptide comprises about 1885 amino acids.

The coding region (open reading frame) of SEQ ID NO:1 is set forth as SEQ ID NO:3. Clone Fbh18610FL, comprising the coding region of human TR-1, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

5

Analysis of the Human TR-1 Molecules

A search using the polypeptide sequence of SEQ ID NO:2 was performed—against the HMM database in PFAM resulting in the identification of three potential transient receptor domains in the amino acid sequence of human TR-1 at about residues
10 699-747, 849-1016, and 1079-1137 of SEQ ID NO:2. A search also identified an ion transport protein domain in the amino acid sequence of human TR-1 (SEQ ID NO:2) at about amino acid residues 884-1096 and an AN1-like zinc finger domain at about residues 33-61.

The amino acid sequence of human TR-1 was analyzed using the program
15 PSORT (<http://www.psort.nibb.ac.jp>) to predict the localization of the proteins within the cell. This program assesses the presence of different targeting and localization amino acid sequences within the query sequence. The results of the analyses show the likelihood of human TR-1 (SEQ ID NO:2) being localized, for example, to the endoplasmic reticulum, the nucleus, and the plasma membrane.

20 A MEMSAT analysis of the polypeptide sequence of SEQ ID NO:2 was also performed, predicting eight potential transmembrane domains in the amino acid sequence of human TR-1 (SEQ ID NO:2) at about residues 282-301, 507-524, 758-774, 856-876, 923-941, 957-974, 1000-1016, and 1127-1146. However, a structural, hydrophobicity, and antigenicity analysis (Figure 2) resulted in the identification of six
25 transmembrane domains and one pore domain between transmembrane domains five and six. These domains are identified in Figure 2 as transmembrane (TM) domains 1 through 6. TM1 is at about residues 758-774, TM2 is at about residues 856-876, TM3 is at about residues 923-941, TM4 is at about residues 957-974, TM5 is at about residues 1000-1016, TM6 is at about residues 1071-1096, and the pore domain is at about
30 residues 1036-1055 of the amino acid sequence set forth as SEQ ID NO:2.

Searches of the amino acid sequence of human TR-1 were further performed against the Prosite database. These searches resulted in the identification in the amino acid sequence of human TR-1 (SEQ ID NO:2) of a number of potential N-glycosylation

sites at about residues 404-407, 550-553, 715-718, 805-808, 925-928, 1058-1061, 1485-1488, 1616-1619, 1794-1797, and 1870-1873, a number of potential cAMP and cGMP-dependent protein kinase phosphorylation sites at about residues 600-603, 754-757, 1493-1496, and 1521-1524, a number of potential kinase C phosphorylation sites at about residues 2-4, 12-14, 22-24, 103-105, 195-197, 318-320, 349-351, 523-525, 529-531, 547-549, 615-617, 697-699, 727-729, 836-838, 842-844, 1245-1247, 1410-1412, 1456-1458, 1491-1493, 1520-1522, 1547-1549, 1719-1721, 1871-1873, and 1880-1882, a number of potential casein kinase II phosphorylation sites at about residues 5-8, 12-15, 22-25, 87-90, 115-118, 299-302, 367-370, 406-409, 508-511, 593-596, 603-606, 675-678, 778-781, 795-798, 883-886, 1163-1166, 1191-1194, 1361-1364, 1413-1416, 1430-1433, 1524-1527, 1547-1550, 1576-1579, 1635-1638, 1652-1655, 1763-1766, 1779-1782, and 1871-1874, a number of potential tyrosine kinase phosphorylation sites at about residues 320-327, 1212-1220, and 1566-1574, a number of potential N-myristoylation sites at about residues 32-37, 99-104, 159-164, 174-179, 208-213, 317-322, 357-362, 402-407, 522-527, 940-945, 1293-1298, 1349-1354, 1385-1390, 1438-1443, 1556-1561, 1642-1647, 1734-1739, and 1790-1795, and an amidation site at about residues 597-600.

A search of the amino acid sequence of human TR-1 (SEQ ID NO:2) was also performed against the ProDom database. The results of this search identified numerous matches against protein domains described as, for example, "receptor from F54D1.5 transient sequence," "melastatin FIS chromosome receptor MTR1 transmembrane," "melastatin receptor chromosome transmembrane transient potential related," "melastatin FIS receptor MTR1 transmembrane chromosome," "receptor channel potential transient NOMPC TRP2 2-beta 2-alpha," "receptor transient potential-related," "channel receptor calcium transient potential repeat vanilloid transmembrane ion transport," "kinase serine/threonine-protein, ATP-binding transferase," "kinase elongation serine/threonine-protein transferase factor-2 eukaryotic calcium/calmodulin-dependent repeat," "kinase receptor-like," and the like were identified.

Tissue Distribution of Human TR-1 mRNA by PCR analysis

This example describes the tissue distribution of human TR-1 mRNA, as may be determined by Polymerase Chain Reaction (PCR) on cDNA libraries using oligonucleotide primers based on the human TR-1 sequence.

5 For *in situ* analysis, various tissues, *e.g.* tissues obtained from brain, are first frozen on dry ice. Ten-micrometer-thick sections of the tissues are postfixed with 4% formaldehyde in DEPC treated 1X phosphate- buffered saline at room temperature for 10 minutes before being rinsed twice in DEPC 1X phosphate-buffered saline and once in 0.1 M triethanolamine-HCl (pH 8.0). Following incubation in 0.25% acetic
10 anhydride-0.1 M triethanolamine-HCl for 10 minutes, sections are rinsed in DEPC 2X SSC (1X SSC is 0.15M NaCl plus 0.015M sodium citrate). Tissue is then dehydrated through a series of ethanol washes, incubated in 100% chloroform for 5 minutes, and then rinsed in 100% ethanol for 1 minute and 95% ethanol for 1 minute and allowed to air dry.

15 Hybridizations are performed with ^{35}S -radiolabeled (5×10^7 cpm/ml) cRNA probes. Probes are incubated in the presence of a solution containing 600 mM NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 0.01% sheared salmon sperm DNA, 0.01% yeast tRNA, 0.05% yeast total RNA type X1, 1X Denhardt's solution, 50% formamide, 10% dextran sulfate, 100 mM dithiothreitol, 0.1% sodium dodecyl sulfate (SDS), and 0.1%
20 sodium thiosulfate for 18 hours at 55°C.

After hybridization, slides are washed with 2X SSC. Sections are then sequentially incubated at 37°C in TNE (a solution containing 10 mM Tris-HCl (pH 7.6), 500 mM NaCl, and 1 mM EDTA), for 10 minutes, in TNE with 10µg of RNase A per ml for 30 minutes, and finally in TNE for 10 minutes. Slides are then rinsed with 2X
25 SSC at room temperature, washed with 2X SSC at 50°C for 1 hour, washed with 0.2X SSC at 55°C for 1 hour, and 0.2X SSC at 60°C for 1 hour. Sections are then dehydrated rapidly through serial ethanol-0.3 M sodium acetate concentrations before being air dried and exposed to Kodak Biomax MR scientific imaging film for 24 hours and subsequently dipped in NB-2 photoemulsion and exposed at 4°C for 7 days before being
30 developed and counter stained.

Tissue Distribution of Human TR-1 mRNA by TaqMan™ analysis

This example describes the tissue distribution of human TR-1 mRNA in a variety of cells and tissues, as determined using the TaqMan™ procedure. The Taqman™ procedure is a quantitative, reverse transcription PCR-based approach for detecting mRNA. The RT-PCR reaction exploits the 5' nuclease activity of AmpliTaq Gold™ DNA Polymerase to cleave a TaqMan™ probe during PCR. Briefly, cDNA was generated from the samples of interest, *e.g.*, various human tissues, and used as the starting material for PCR amplification. In addition to the 5' and 3' gene-specific primers, a gene-specific oligonucleotide probe (complementary to the region being amplified) was included in the reaction (*i.e.*, the Taqman™ probe). The TaqMan™ probe includes the oligonucleotide with a fluorescent reporter dye covalently linked to the 5' end of the probe (such as FAM (6-carboxyfluorescein), TET (6-carboxy-4,7,2',7'-tetrachlorofluorescein), JOE (6-carboxy-4,5-dichloro-2,7-dimethoxyfluorescein), or VIC) and a quencher dye (TAMRA (6-carboxy-N,N,N',N'-tetramethylrhodamine) at the 3' end of the probe.

During the PCR reaction, cleavage of the probe separates the reporter dye and the quencher dye, resulting in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'-3' nucleolytic activity of the AmpliTaq™ Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. RNA was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA. cDNA was synthesized using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no detectable PCR amplification of the control gene confirms efficient removal of genomic DNA contamination.

A human tissue panel was tested revealing highest expression of human TR-1 mRNA in the in Jurkat cells (T-cell leukemia cells) and K562 cells (chronic myeloid leukemia cells) (see Figure 3), indicating a role for TR-1 in cellular proliferation, growth, differentiation, or migration disorders such as cancer.

5

EXAMPLE 2: EXPRESSION OF RECOMBINANT TR-1 POLYPEPTIDE IN BACTERIAL CELLS

10 In this example, human TR-1 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, TR-1 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-TR-1 fusion polypeptide in PEB199 is induced with IPTG. The recombinant fusion polypeptide is
15 purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

20

EXAMPLE 3: EXPRESSION OF RECOMBINANT TR-1 POLYPEPTIDE IN COS CELLS

To express the human TR-1 gene in COS cells, the pcDNA/Amp vector by
25 Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire TR-1 polypeptide and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the
30 polylinker region of the vector, thereby placing the expression of the recombinant polypeptide under the control of the CMV promoter.

To construct the plasmid, the human TR-1 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the TR-1 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the TR-1 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the TR-1 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5 α , SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the human TR-1-pCDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the IC54420 polypeptide is detected by radiolabelling (^{35}S -methionine or ^{35}S -cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labelled for 8 hours with ^{35}S -methionine (or ^{35}S -cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the human TR-1 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above,

and the expression of the TR-1 polypeptide is detected by radiolabelling and immunoprecipitation using a TR-1-specific monoclonal antibody.

Equivalents

- 5 Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1. An isolated nucleic acid molecule selected from the group consisting of:
 - 5 (a) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:1; and
 - (b) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:3.
- 10 2. An isolated nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2.
3. An isolated nucleic acid molecule comprising the nucleotide sequence contained in the plasmid deposited with ATCC® as Accession Number ____.
- 15 4. An isolated nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2.
- 20 5. An isolated nucleic acid molecule selected from the group consisting of:
 - a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof;
 - 25 b) a nucleic acid molecule comprising a fragment of at least 100 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof;
 - c) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ
 - 30 ID NO:2; and

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 10 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2.

5

6. An isolated nucleic acid molecule which hybridizes to a complement of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 under stringent conditions.

7. An isolated nucleic acid molecule comprising a nucleotide sequence
10 which is complementary to the nucleotide sequence of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5.

8. An isolated nucleic acid molecule comprising the nucleic acid molecule
of any one of claims 1, 2, 3, 4, or 5, and a nucleotide sequence encoding a heterologous
15 polypeptide.

9. A vector comprising the nucleic acid molecule of any one of claims 1, 2,
3, 4, or 5.

20 10. The vector of claim 9, which is an expression vector.

11. A host cell transfected with the expression vector of claim 10.

12. A method of producing a polypeptide comprising culturing the host cell
25 of claim 11 in an appropriate culture medium to, thereby, produce the polypeptide.

13. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence
30 of SEQ ID NO:2, wherein the fragment comprises at least 10 contiguous amino acids of
SEQ ID NO:2;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 or 3 under stringent conditions;

5 c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3; and

d) a polypeptide comprising an amino acid sequence which is at least 60% identical to the amino acid sequence of SEQ ID NO:2.

10

14. The isolated polypeptide of claim 13 comprising the amino acid sequence of SEQ ID NO:2.

15. The polypeptide of claim 13, further comprising heterologous amino acid
15 sequences.

16. An antibody which selectively binds to a polypeptide of claim 13.

17. A method for detecting the presence of a polypeptide of claim 13 in a
20 sample comprising:

a) contacting the sample with a compound which selectively binds to the polypeptide; and

b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 13 in the sample.

25

18. The method of claim 17, wherein the compound which binds to the polypeptide is an antibody.

19. A kit comprising a compound which selectively binds to a polypeptide of
30 claim 13 and instructions for use.

20. A method for detecting the presence of a nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in a sample comprising:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to a complement of the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to the complement of the nucleic acid molecule in the sample to thereby detect the presence of
5 the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in the sample.

21. The method of claim 20, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

10 22. A kit comprising a compound which selectively hybridizes to a complement of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 and instructions for use.

23. A method for identifying a compound which binds to a polypeptide of
15 claim 13 comprising:

a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and

b) determining whether the polypeptide binds to the test compound.

20 24. The method of claim 23, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detection of test compound/polypeptide binding;

b) detection of binding using a competition binding assay; and

25 c) detection of binding using an assay for TR-1 activity.

25. A method for modulating the activity of a polypeptide of claim 13 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the
30 activity of the polypeptide.

26. A method for identifying a compound which modulates the activity of a polypeptide of claim 13 comprising:

- a) contacting a polypeptide of claim 13 with a test compound; and
- b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide..

5

M S Q K S W I E S T L T K R																		14
GAGTCCGCCCCGTGAGGAGA ATG TCC CAG AAA TCC TGG ATA GAA AGC ACT TTG ACC AAG AGG																		42
E C V Y I I P S S K D P H R C L P G C Q	34																	
GAA TGT GTA TAT ATT ATA CCA AGT TCC AAG GAC CCT CAC AGA TGC CTT CCA GGA TGT CAA	102																	
I C Q Q L V R C F C G R L V K Q H A C F	54																	
ATT TGT CAG CAA CTC GTC AGG TGT TTT TGT GGT CGC TTG GTC AAG CAA CAT GCT TGT TTT	162																	
T A S L A M K Y S D V K L G D H F N Q A	74																	
ACT GCA AGT CTT GCC ATG AAA TAC TCA GAT GTG AAA TTG GGT GAC CAT TTT AAT CAG GCA	222																	
I E E W S V E K H T E Q S P T D A Y G V	94																	
ATA GAA GAA TGG TCT GTG GAA AAG CAT ACA GAA CAG AGC CCA ACG GAT GCT TAT GGA GTC	282																	
I N F Q G G S H S Y R A K Y V R L S Y D	114																	
ATA AAT TTT CAA GGG GGT TCT CAT TCC TAC AGA GCT AAG TAT GTG AGG CTA TCA TAT GAC	342																	
T K P E V I L Q L L L K E W Q M E L P K	134																	
ACC AAA CCT GAA GTC ATT CTG CAA CTT CTG CTT AAA GAA TGG CAA ATG GAG TTA CCC AAA	402																	
L V I S V H G G M Q K F E L H P R I K Q	154																	
CTT GTT ATC TCT GTA CAT GGG GGC ATG CAG AAA TTT GAG CTT CAC CCA CGA ATC AAG CAG	462																	
L L G K G L I K A A V T T G A W I L T G	174																	
TTG CTT GGA AAA GGT CTT ATT AAA GCT GCA GTT ACA ACT GGA GCC TGG ATT TTA ACT GGA	522																	
G V N T G V A K H V G D A L K E H A S R	194																	
GGA GTA AAC ACA GGT GTG GCA AAA CAT GTT GGA GAT GCC CTC AAA GAA CAT GCT TCC AGA	582																	
S S R K I C T I G I A P W G V I E N R N	214																	
TCA TCT CGA AAG ATT TGC ACT ATC GGA ATA GCT CCA TGG GGA GTG ATT GAA AAC AGA AAT	642																	
D L V G R D V V A P Y Q T L L N P L S K	234																	
GAT CTT GTT GGG AGA GAT GTG GTT GCT CCT TAT CAA ACC TTA TTG AAC CCC CTG AGC AAA	702																	
L N V L N N L H S H F I L V D D G T V G	254																	
TTG AAT GTT TTG AAT AAT CTG CAT TCC CAT TTC ATA TTG GTG GAT GAT GGC ACT GTT GGA	762																	
K Y G A E V R L R R E L E K T I N Q Q R	274																	
AAG TAT GGG GCG GAA GTC AGA CTG AGA AGA GAA CTT GAA AAA ACT ATT AAT CAG CAA AGA	822																	
I H A R I G Q G V P V V A L I F E G G P	294																	
ATT CAT GCT AGG ATT GGC CAG GGT GTC CCT GTG GTG GCA CTT ATA TTT GAG GGT GGG CCA	882																	
N V I L T V L E Y L Q E S P P V P V V V	314																	
AAT GTT ATC CTC ACA GTT CTT GAA TAC CTT CAG GAA AGC CCC CCT GTT CCA GTA GTT GTG	942																	
C E G T G R A A D L L A Y I H K Q T E E	334																	
TGT GAA GGA ACA GGC AGA GCT GCA GAT CTG CTA GCG TAT ATT CAT AAA CAA ACA GAA GAA	1002																	
G G N L P D A A G H D I I S T I K K T F	354																	
GGA GGG AAT CTT CCT GAT GCA GCA GGG CAC GAT ATT ATT TCC ACT ATC AAA AAA ACA TTT	1062																	
N F G Q N E A L H L F Q T L M E C M K R	374																	

BNSDOCID: <WO__0210391A2_I_>

AAC TTT GGC CAG AAT GAA GCA CTT CAT TTA TTT CAA ACA CTG ATG GAG TGC ATG AAA AGA 1122
 K E L I T V F H I G S D E H Q D I D V A 394
 AAG GAG CTT ATC ACT GTT TTC CAT ATT GGG TCA GAT GAA CAT CAA GAT ATA GAT GTA GCA 1182
 I L T A L L K G T N A S A F D Q L I L T 414
 ATA CTT ACT GCA CTG CTA AAA GGT ACT AAT GCA TCT GCA TTT GAC CAG CTT ATC CTT ACA 1242
 L A W G R V D I A K N H V F V Y G Q Q W 434
 TTG GCA TGG GGT AGA GTT GAC ATT GCC AAA AAT CAT GTA TTT GTT TAT GGA CAG CAG TGG 1302
 L V G S L E Q A M L D A L V M D R V A F 454
 CTG GTT GGA TCC TTG GAA CAA GCT ATG CTT GAT GCT CTT GTA ATG GAT AGA GTT GCA TTT 1362
 V K L L I E N G V S M H K F L T I P R L 474
 GTA AAA CTT CTT ATT GAA AAT GGA GTA AGC ATG CAT AAA TTC CTT ACC ATT CCG AGA CTG 1422
 E E L Y N T K Q G P T N P M L F H L V R 494
 GAA GAA CTT TAC AAC ACT AAA CAA GGT CCA ACT AAT CCA ATG CTG TTT CAT CTT GTT CGA 1482
 D V K Q G N L P P G Y K I A L I D I G L 514
 GAC GTC AAA CAG GGA AAT CTT CCT CCA GGA TAT AAG ATC GCT CTG ATT GAT ATA GGA CTT 1542
 V I E Y L M G G T Y R C T Y T R K R F R 534
 GTT ATT GAA TAT CTC ATG GGA GGA ACC TAC AGA TGC ACC TAT ACT AGG AAA CGT TTT CGA 1602
 L I Y N S L G G N N R R S G R N T S S S 554
 TTA ATA TAT AAT AGT CTT GGT GGA AAT AAT CGG AGG TCT GGC CGA AAT ACC TCC AGC AGC 1662
 T P Q L R K S H E F F G N R A D K K E K 574
 ACT CCT CAG TTG CGA AAG AGT CAT GAA TTT TTT GGC AAT AGG GCA GAT AAA AAG GAA AAA 1722
 M R H N H F I K T A Q P Y R P K I D T V 594
 ATG AGG CAT AAC CAT TTC ATT AAG ACA GCA CAG CCC TAC CGA CCA AAG ATT GAT ACA GTT 1782
 M E E G K K K R T K D E I V D I D D P E 614
 ATG GAA GAA GGA AAG AAG AAA AGA ACC AAA GAT GAA ATT GTA GAC ATT GAT GAT CCA GAA 1842
 T K R F P Y P L N E L L I W A C L M K R 634
 ACC AAG CGC TTT CCT TAT CCA CTT AAT GAA CTT TTA ATT TGG GCT TGC CTT ATG AAG AGG 1902
 Q V M A R F L W Q H G E E S M A K A L V 654
 CAG GTC ATG GCC CGT TTT TTA TGG CAA CAT GGT GAA GAA TCA ATG GCT AAA GCA TTA GTT 1962
 A C K I Y R S M A Y E A K Q S D L V D D 674
 GCC TGT AAG ATC TAT CGT TCA ATG GCA TAT GAA GCA AAG CAG AGT GAC CTG GTA GAT GAT 2022
 T S E E L K Q Y S N D F G Q L A V E L L 694
 ACT TCA GAA GAA CTA AAA CAG TAT TCC AAT GAT TTT GGT CAG TTG GCC GTT GAA TTA TTA 2082
 E Q S F R Q D E T M A M K L L T Y E L K 714
 GAA CAG TCC TTC AGA CAA GAT GAA ACC ATG GCT ATG AAA TTG CTC ACT TAT GAA CTG AAG 2142
 N W S N S T C L K L A V S S R L R P F V 734
 AAC TGG AGT AAT TCA ACC TGC CTT AAG TTA GCA GTT TCT TCA AGA CTT AGA CCT TTT GTA 2202
 A H T C T Q M L L S D M W M G R L N M R 754
 GCT CAC ACC TGT ACA CAA ATG TTG TTA TCT GAT ATG TGG ATG GGA AGG CTG AAT ATG AGG 2262
 K N S W Y K V I L S I L V P P A I L L L 774
 AAA AAT TCC TGG TAC AAG GTC ATA CTA AGC ATT TTA GTT CCA CCT GCC ATA TTG CTG TTA 2322
 E Y K T K A E M S H I P Q S Q D A H Q M 794
 GAG TAT AAA ACT AAG GCT GAA ATG TCC CAT ATC CCA CAA TCT CAA GAT GCT CAT CAG ATG 2382

FIGURE 1B

T	M	D	D	S	E	N	N	F	Q	N	I	T	E	E	I	P	M	E	V	814
ACA	ATG	GAT	GAC	AGC	GAA	AAC	AAC	TTT	CAG	AAC	ATA	ACA	GAA	GAG	ATC	CCC	ATG	GAA	GTG	2442
F	K	E	V	R	I	L	D	S	N	E	G	K	N	E	M	E	I	Q	M	834
TTT	AAA	GAA	GTA	CGG	ATT	TTG	GAT	AGT	AAT	GAA	GGA	AAG	AAT	GAG	ATG	GAG	ATA	CAA	ATG	2502
K	S	K	K	L	P	I	T	R	K	F	Y	A	F	Y	H	A	P	I	V	854
AAA	TCA	AAA	AAG	CTT	CCA	ATT	ACG	CGA	AAG	TTT	TAT	GCC	TTT	TAT	CAT	GCA	CCA	ATT	GTA	2562
K	F	W	F	N	T	L	A	Y	L	G	F	L	M	L	Y	T	F	V	V	874
AAA	TTC	TGG	TTT	AAC	ACG	TTG	GCA	TAT	TTA	GGA	TTT	CTG	ATG	CTT	TAT	ACA	TTT	GTG	GTT	2622
L	V	Q	M	E	Q	L	P	S	V	Q	E	W	I	V	I	A	Y	I	F	894
CTT	GTA	CAA	ATG	GAA	CAG	TTA	CCT	TCA	GTT	CAA	GAA	TGG	ATT	GTT	ATT	GCT	TAT	ATT	TTT	2682
T	Y	A	I	E	K	V	R	E	I	F	M	S	E	A	G	K	V	N	Q	914
ACT	TAT	GCC	ATT	GAG	AAA	GTC	CGT	GAG	ATC	TTT	ATG	TCT	GAA	GCT	GGG	AAA	GTA	AAC	CAG	2742
K	I	K	V	W	F	S	D	Y	F	N	I	S	D	T	I	A	I	I	S	934
AAG	ATT	AAA	GTA	TGG	TTT	AGT	GAT	TAC	TTC	AAC	ATC	AGT	GAT	ACA	ATT	GCC	ATA	ATT	TCT	2802
F	F	I	G	F	G	L	R	F	G	A	K	W	N	F	A	N	A	Y	D	954
TTC	TTC	ATT	GGA	TTT	GGA	CTA	AGA	TTT	GGA	GCA	AAA	TGG	AAC	TTT	GCA	AAT	GCA	TAT	GAT	2862
N	H	V	F	V	A	G	R	L	I	Y	C	L	N	I	I	F	W	Y	V	974
AAT	CAT	GTT	TTT	GTG	GCT	GGA	AGA	TTA	ATT	TAC	TGT	CTT	AAC	ATA	ATA	TTT	TGG	TAT	GTG	2922
R	L	L	D	F	L	A	V	N	Q	Q	A	G	P	Y	V	M	M	I	G	994
CGT	TTG	CTA	GAT	TTT	CTA	GCT	GTA	AAT	CAA	CAG	GCA	GGA	CCT	TAT	GTA	ATG	ATG	ATT	GGA	2982
K	M	V	A	N	M	F	Y	I	V	V	I	M	A	L	V	L	L	S	F	1014
AAA	ATG	GTG	GCC	AAT	ATG	TTC	TAC	ATT	GTA	GTG	ATT	ATG	GCT	CTT	GTA	TTA	CTT	AGT	TTT	3042
G	V	P	R	K	A	I	L	Y	P	H	E	A	P	S	W	T	L	A	K	1034
GGT	GTT	CCC	AGA	AAG	GCA	ATA	CTT	TAT	CCT	CAT	GAA	GCA	CCA	TCT	TGG	ACT	CTT	GCT	AAA	3102
D	I	V	F	H	P	Y	W	M	I	F	G	E	V	Y	A	Y	E	I	D	1054
GAT	ATA	GTT	TTT	CAC	CCA	TAC	TGG	ATG	ATT	TTT	GGT	GAA	GTT	TAT	GCA	TAC	GAA	ATT	GAT	3162
V	C	A	N	D	S	V	I	P	Q	I	C	G	P	G	T	W	L	T	P	1074
GTG	TGT	GCA	AAT	GAT	TCT	GTT	ATC	CCT	CAA	ATC	TGT	GGT	CCT	GGG	ACG	TGG	TTG	ACT	CCA	3222
F	L	Q	A	V	Y	L	F	V	Q	Y	I	I	M	V	N	L	L	I	A	1094
TTT	CTT	CAA	GCA	GTC	TAC	CTC	TTT	GTA	CAG	TAT	ATC	ATT	ATG	GTT	AAT	CTT	CTT	ATT	GCA	3282
F	F	N	N	V	Y	L	Q	V	K	A	I	S	N	I	V	W	K	Y	Q	1114
TTT	TTC	AAC	AAT	GTG	TAT	TTA	CAA	GTG	AAG	GCA	ATT	TCC	AAT	ATT	GTA	TGG	AAG	TAC	CAG	3342
R	Y	H	F	I	M	A	Y	H	E	K	P	V	L	P	P	P	L	I	I	1134
CGT	TAT	CAT	TTT	ATT	ATG	GCT	TAT	CAT	GAG	AAA	CCA	GTT	CTG	CCT	CCT	CCA	CTT	ATC	ATT	3402
L	S	H	I	V	S	L	F	C	C	I	C	K	R	R	K	K	D	K	T	1154
CTT	AGC	CAT	ATA	GTT	TCT	CTG	TTT	TGC	TGC	ATA	TGT	AAG	AGA	AGA	AAG	AAA	GAT	AAG	ACT	3462
S	D	G	P	K	L	F	L	T	E	E	D	Q	K	K	L	H	D	F	E	1174
TCC	GAT	GGA	CCA	AAA	CTT	TTC	TTA	ACA	GAA	GAA	GAT	CAA	AAG	AAA	CTT	CAT	GAT	TTT	GAA	3522
E	Q	C	V	E	M	Y	F	N	E	K	D	D	K	F	H	S	G	S	E	1194
GAG	CAG	TGT	GTT	GAA	ATG	TAT	TTC	AAT	GAA	AAA	GAT	GAC	AAA	TTT	CAT	TCT	GGG	AGT	GAA	3582
E	R	I	R	V	T	F	E	R	V	E	Q	M	C	I	Q	I	K	E	V	1214
GAG	AGA	ATT	CGT	GTC	ACT	TTT	GAA	AGA	GTG	GAA	CAG	ATG	TGC	ATT	CAG	ATT	AAA	GAA	GTT	3642

FIGURE 1C

G	D	R	V	N	Y	I	K	R	S	L	Q	S	L	D	S	Q	I	G	H	1234
GGA	GAT	CGT	GTC	AAC	TAC	ATA	AAA	AGA	TCA	TTA	CAA	TCA	TTA	GAT	TCT	CAA	ATT	GGC	CAT	3702
L	Q	D	L	S	A	L	T	V	D	T	L	K	T	L	T	A	Q	K	A	1254
TTG	CAA	GAT	CTT	TCA	GCC	CTG	ACG	GTA	GAT	ACA	TTA	AAA	ACA	CTC	ACT	GCC	CAG	AAA	GCG	3762
S	E	A	S	K	V	H	N	E	I	T	R	E	L	S	I	S	K	H	L	1274
TCG	GAA	GCT	AGC	AAA	GTT	CAT	AAT	GAA	ATC	ACA	CGA	GAA	CTG	AGC	ATT	TCC	AAA	CAC	TTG	3822
A	Q	N	L	I	D	D	G	P	V	R	P	S	V	W	K	K	H	G	V	1294
GCT	CAA	AAC	CTT	ATT	GAT	GAT	GGT	CCT	GTA	AGA	CCT	TCT	GTA	TGG	AAA	AAG	CAT	GGT	GTT	3882
V	N	T	L	S	S	S	L	P	Q	G	D	L	E	S	N	N	P	F	H	1314
GTA	AAT	ACA	CTT	AGC	TCC	TCT	CTT	CCT	CAA	GGT	GAT	CTT	GAA	AGT	AAT	AAT	CCT	TTT	CAT	3942
C	N	I	L	M	K	D	D	K	D	P	Q	C	N	I	F	G	Q	D	L	1334
TGT	AAT	ATT	TTA	ATG	AAA	GAT	GAC	AAA	GAT	CCC	CAG	TGT	AAT	ATA	TTT	GGT	CAA	GAC	TTA	4002
P	A	V	P	Q	R	K	E	F	N	F	P	E	A	G	S	S	S	G	A	1354
CCT	GCA	GTA	CCC	CAG	AGA	AAA	GAA	TTT	AAT	TTT	CCA	GAG	GCT	GGT	TCC	TCT	TCT	GGT	GCC	4062
L	F	P	S	A	V	S	P	P	E	L	R	Q	R	L	H	G	V	E	L	1374
TTA	TTC	CCA	AGT	GCT	GTT	TCC	CCT	CCA	GAA	CTG	CGA	CAG	AGA	CTA	CAT	GGG	GTA	GAA	CTC	4122
L	K	I	F	N	K	N	Q	K	L	G	S	S	S	T	S	I	P	H	L	1394
TTA	AAA	ATA	TTT	AAT	AAA	AAT	CAA	AAA	TTA	GGC	AGT	TCA	TCT	ACT	AGC	ATA	CCA	CAT	CTG	4182
S	S	P	P	T	K	F	F	V	S	T	P	S	Q	P	S	C	K	S	H	1414
TCA	TCC	CCA	CCA	ACC	AAA	TTT	TTT	GTT	AGT	ACA	CCA	TCT	CAG	CCA	AGT	TGC	AAA	AGC	CAC	4242
L	E	T	G	T	K	D	Q	E	T	V	C	S	K	A	T	E	G	D	N	1434
TTG	GAA	ACT	GGA	ACC	AAA	GAT	CAA	GAA	ACT	GTT	TGC	TCT	AAA	GCT	ACA	GAA	GGA	GAT	AAT	4302
T	E	F	G	A	F	V	G	H	R	D	S	M	D	L	Q	R	F	K	E	1454
ACA	GAA	TTT	GGA	GCA	TTT	GTA	GGA	CAC	AGA	GAT	AGC	ATG	GAT	TTA	CAG	AGG	TTT	AAA	GAA	4362
T	S	N	K	I	K	I	L	S	E	Q	Q	N	D	V	R	N	V	I	M	1474
ACA	TCA	AAC	AAG	ATA	AAA	ATA	CTA	TCC	GAG	CAG	CAG	AAT	GAT	GTA	AGA	AAT	GTG	ATT	ATG	4422
E	Y	T	E	M	P	K	Y	E	N	N	N	T	S	E	N	T	L	K	R	1494
GAG	TAT	ACA	GAG	ATG	CCT	AAA	TAT	GAG	AAT	AAC	AAT	ACT	TCT	GAA	AAC	ACT	TTG	AAA	CGA	4482
V	S	S	L	A	G	F	T	D	C	H	R	T	S	I	P	V	H	S	K	1514
GTG	AGT	TCT	CTT	GCT	GGA	TTT	ACT	GAC	TGT	CAC	AGA	ACT	TCC	ATT	CCT	GTT	CAT	TCA	AAA	4542
Q	A	E	K	I	S	R	R	P	S	T	E	D	T	H	E	V	D	S	K	1534
CAA	GCA	GAA	AAA	ATC	AGT	AGA	AGG	CCA	TCT	ACC	GAA	GAC	ACT	CAT	GAA	GTA	GAT	TCC	AAA	4602
A	A	L	I	P	D	W	L	Q	D	R	P	S	N	R	E	M	P	S	E	1554
GCA	GCT	TTA	ATA	CCG	GAT	TGG	TTA	CAA	GAT	AGA	CCA	TCA	AAC	AGA	GAA	ATG	CCA	TCT	GAA	4662
E	G	T	L	N	G	L	T	S	P	F	K	P	A	M	D	T	N	Y	Y	1574
GAA	GGA	ACA	TTA	AAT	GGT	CTC	ACT	TCT	CCA	TTT	AAG	CCA	GCT	ATG	GAT	ACA	AAT	TAC	TAT	4722
Y	S	A	V	E	R	N	N	L	M	R	L	S	Q	S	I	P	F	T	P	1594
TAT	TCA	GCT	GTG	GAA	AGA	AAT	AAC	TTG	ATG	AGG	TTA	TCA	CAG	AGC	ATT	CCA	TTT	ACA	CCT	4782
V	P	P	R	G	E	P	V	T	V	Y	R	L	E	E	S	S	P	N	I	1614
GTG	CCT	CCA	AGA	GGG	GAG	CCT	GTC	ACA	GTG	TAT	CGT	TTG	GAA	GAG	AGT	TCA	CCC	AAC	ATA	4842
L	N	N	S	M	S	S	W	S	Q	L	G	L	C	A	K	I	E	F	L	1634
CTA	AAT	AAC	AGC	ATG	TCT	TCT	TGG	TCA	CAA	CTA	GGC	CTC	TGT	GCC	AAA	ATA	GAG	TTT	TTA	4902
S	K	E	E	M	G	G	G	L	R	R	A	V	K	V	Q	C	T	W	S	1654

FIGURE 1D

AGC AAA GAG GAG ATG GGA GGA GGT TTA CGA AGA GCT GTC AAA GTA CAG TGT ACC TGG TCA 4962
 E H D I L K S G H L Y I I K S F L P E V 1674
 GAA CAT GAT ATC CTC AAA TCA GGG CAT CTT TAT ATT ATC AAA TCT TTT CTT CCA GAG GTG 5022
 V N T W S S I Y K E D T V L H L C L R E 1694
 GTT AAT ACA TGG TCA AGT ATT TAT AAA GAA GAT ACA GTT CTG CAT CTC TGT CTG AGA GAA 5082
 I Q Q Q R A A Q K L T F A F N Q M K P K 1714
 ATT CAA CAA CAG AGA GCA GCA CAA AAG CTT ACG TTT GCC TTT AAT CAA ATG AAA CCC AAA 5142
 S I P Y S P R F L E V F L L Y C H S A G 1734
 TCC ATA CCA TAT TCT CCA AGG TTC CTT GAA GTT TTC CTG CTG TAT TGC CAT TCA GCA GGA 5202
 Q W F A V E E C M T G E F R K Y N N N N 1754
 CAG TGG TTT GCT GTG GAA GAA TGT ATG ACT GGA GAA TTT AGA AAA TAC AAC AAT AAT AAT 5262
 G D E I I P T N T L E E I M L A F S H W 1774
 GGA GAT GAG ATT ATT CCA ACT AAT ACT CTG GAA GAG ATC ATG CTA GCC TTT AGC CAC TGG 5322
 T Y E Y T R G E L L V L D L Q G V G E N 1794
 ACT TAC GAA TAT ACA AGA GGG GAG TTA CTG GTA CTT GAT TTG CAA GGT GTT GGT GAA AAT 5382
 L T D P S V I K A E E K R S C D M V F G 1814
 TTG ACT GAC CCA TCT GTG ATA AAA GCA GAA GAA AAG AGA TCC TGT GAT ATG GTT TTT GGC 5442
 P A N L G E D A I K N F R A K H H C N S 1834
 CCA GCA AAT CTA GGA GAA GAT GCA ATT AAA AAC TTC AGA GCA AAA CAT CAC TGT AAT TCT 5502
 C C R K L K L P D L K R N D Y T P D K I 1854
 TGC TGT AGA AAG CTT AAA CTT CCA GAT CTG AAG AGG AAT GAT TAT ACG CCT GAT AAA ATT 5562
 I F P Q D E P S D L N L Q P G N S T K E 1874
 ATA TTT CCT CAG GAT GAG CCT TCA GAT TTG AAT CTT CAG CCT GGA AAT TCC ACC AAA GAA 5622
 S E S T N S V R L M L * 1886
 TCA GAA TCA ACT AAT TCT GTT CGT CTG ATG TTA TAA 5658
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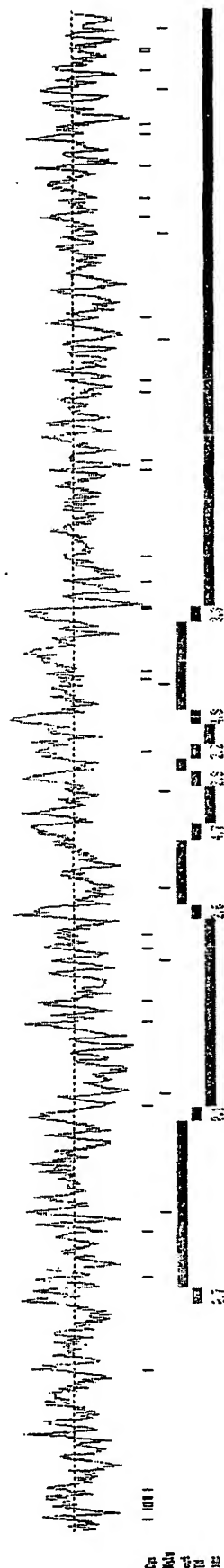
FIGURE 1E

CCTTATGCCAATAAAATGTAATTTAACAGCCCCAGATATTGTTGAATATTCAACAATAACAAGAAAAGCTTTTCATCTA
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ATAGTGTTAATATTGATTGGGGGTGGGAGAAAGAAAAGTGTATTACTTAAAGATACTATATACGTTTTGTATATCA
TTAAATCTTTAAAAGAAATGAAATAAATTTATTGTTTACAGATGAAAAAAAAAAAAAAAAAAAAAGGGCGGCCGC

FIGURE 1F

FIG. 2

100 Hz



1 40 80 120 160 200 240 280 320 360 400 440 480 520 560 600 640 680 720 760 800 840 880 920 960 1000 1040 1080 1120 1160 1200 1240 1280 1320 1360 1400 1440 1480 1520 1560 1600 1640 1680 1720 1760 1800 1840 1880

FIGURE 2

18610 Expression

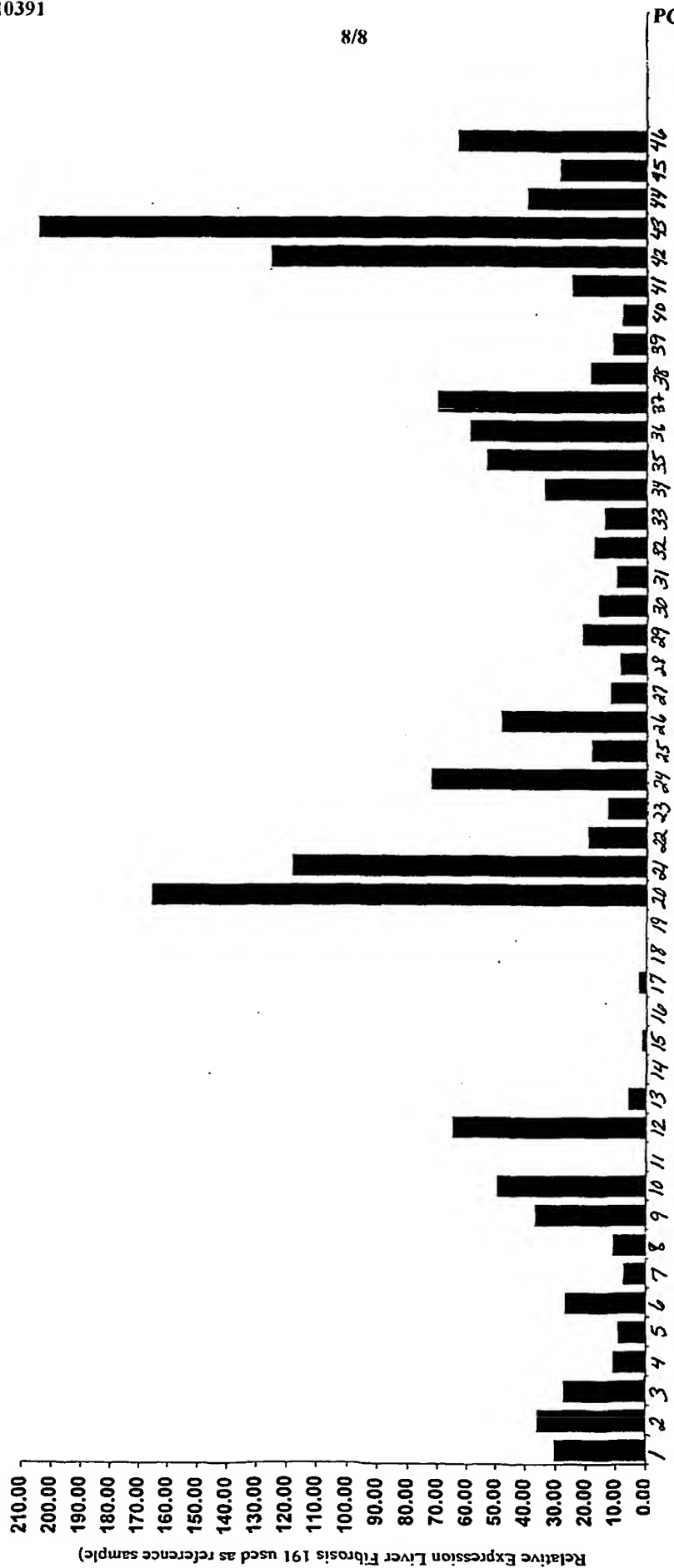


FIGURE 3

SEQUENCE LISTING

<110> Millennium Pharmaceuticals, Inc., et al.

<120> 18610, A NOVEL HUMAN TRANSIENT RECEPTOR
AND USES THEREOF

<130> MNI-182PC

<150> USSN 60/221,925

<151> 2000-07-31

<160> 3

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 7334

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (258)...(5912)

<400> 1

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gagaagggttt ccctgggcgt tccttgctcg gcggcctctg ctgccgcctc cggagacgct 180
tcccgataga tggctacagg ccgcggagga ggaggagggtg gagttgctgc ccttcgggag 240
tccgcccgct gaggaga atg tcc cag aaa tcc tgg ata gaa agc act ttg      290
                Met Ser Gln Lys Ser Trp Ile Glu Ser Thr Leu
                  1             5             10

acc aag agg gaa tgt gta tat att ata cca agt tcc aag gac cct cac      338
Thr Lys Arg Glu Cys Val Tyr Ile Ile Pro Ser Ser Lys Asp Pro His
              15             20             25

aga tgc ctt cca gga tgt caa att tgt cag caa ctc gtc agg tgt ttt      386
Arg Cys Leu Pro Gly Cys Gln Ile Cys Gln Gln Leu Val Arg Cys Phe
              30             35             40

tgt ggt cgc ttg gtc aag caa cat gct tgt ttt act gca agt ctt gcc      434
Cys Gly Arg Leu Val Lys Gln His Ala Cys Phe Thr Ala Ser Leu Ala
              45             50             55

atg aaa tac tca gat gtg aaa ttg ggt gac cat ttt aat cag gca ata      482
Met Lys Tyr Ser Asp Val Lys Leu Gly Asp His Phe Asn Gln Ala Ile
              60             65             70             75

gaa gaa tgg tct gtg gaa aag cat aca gaa cag agc cca acg gat gct      530
Glu Glu Trp Ser Val Glu Lys His Thr Glu Gln Ser Pro Thr Asp Ala
              80             85             90

tat gga gtc ata aat ttt caa ggg ggt tct cat tcc tac aga gct aag      578
Tyr Gly Val Ile Asn Phe Gln Gly Gly Ser His Ser Tyr Arg Ala Lys
              95             100            105

tat gtg agg cta tca tat gac acc aaa cct gaa gtc att ctg caa ctt      626
Tyr Val Arg Leu Ser Tyr Asp Thr Lys Pro Glu Val Ile Leu Gln Leu
              110            115            120

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ctg ctt aaa gaa tgg caa atg gag tta ccc aaa ctt gtt atc tct gta	674
Leu Leu Lys Glu Trp Gln Met Glu Leu Pro Lys Leu Val Ile Ser Val	
125 130 135	
cat ggg ggc atg cag aaa ttt gag ctt cac cca cga atc aag cag ttg	722
His Gly Gly Met Gln Lys Phe Glu Leu His Pro Arg Ile Lys Gln Leu	
140 145 150 155	
ctt gga aaa ggt ctt att aaa gct gca gtt aca act gga gcc tgg att	770
Leu Gly Lys Gly Leu Ile Lys Ala Ala Val Thr Thr Gly Ala Trp Ile	
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tta act gga gga gta aac aca ggt gtg gca aaa cat gtt gga gat gcc	818
Leu Thr Gly Gly Val Asn Thr Gly Val Ala Lys His Val Gly Asp Ala	
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Ile Ala Pro Trp Gly Val Ile Glu Asn Arg Asn Asp Leu Val Gly Arg	
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Asp Val Val Ala Pro Tyr Gln Thr Leu Leu Asn Pro Leu Ser Lys Leu	
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Asn Val Leu Asn Asn Leu His Ser His Phe Ile Leu Val Asp Asp Gly	
240 245 250	
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Thr Val Gly Lys Tyr Gly Ala Glu Val Arg Leu Arg Arg Glu Leu Glu	
255 260 265	
aaa act att aat cag caa aga att cat gct agg att ggc cag ggt gtc	1106
Lys Thr Ile Asn Gln Gln Arg Ile His Ala Arg Ile Gly Gln Gly Val	
270 275 280	
cct gtg gtg gca ctt ata ttt gag ggt ggg cca aat gtt atc ctc aca	1154
Pro Val Val Ala Leu Ile Phe Glu Gly Gly Pro Asn Val Ile Leu Thr	
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Glu Gly Thr Gly Arg Ala Ala Asp Leu Leu Ala Tyr Ile His Lys Gln	
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Thr Glu Glu Gly Gly Asn Leu Pro Asp Ala Ala Gly His Asp Ile Ile	
335 340 345	
tcc act atc aaa aaa aca ttt aac ttt ggc cag aat gaa gca ctt cat	1346
Ser Thr Ile Lys Lys Thr Phe Asn Phe Gly Gln Asn Glu Ala Leu His	
350 355 360	

tta ttt caa aca ctg atg gag tgc atg aaa aga aag gag ctt atc act	1394
Leu Phe Gln Thr Leu Met Glu Cys Met Lys Arg Lys Glu Leu Ile Thr	
365 370 375	
gtt ttc cat att ggg tca gat gaa cat caa gat ata gat gta gca ata	1442
Val Phe His Ile Gly Ser Asp Glu His Gln Asp Ile Asp Val Ala Ile	
380 385 390 395	
ctt act gca ctg cta aaa ggt act aat gca tct gca ttt gac cag ctt	1490
Leu Thr Ala Leu Leu Lys Gly Thr Asn Ala Ser Ala Phe Asp Gln Leu	
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atc ctt aca ttg gca tgg ggt aga gtt gac att gcc aaa aat cat gta	1538
Ile Leu Thr Leu Ala Trp Gly Arg Val Asp Ile Ala Lys Asn His Val	
415 420 425	
ttt gtt tat gga cag cag tgg ctg gtt gga tcc ttg gaa caa gct atg	1586
Phe Val Tyr Gly Gln Gln Trp Leu Val Gly Ser Leu Glu Gln Ala Met	
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ctt gat gct ctt gta atg gat aga gtt gca ttt gta aaa ctt ctt att	1634
Leu Asp Ala Leu Val Met Asp Arg Val Ala Phe Val Lys Leu Leu Ile	
445 450 455	
gaa aat gga gta agc atg cat aaa ttc ctt acc att ccg aga ctg gaa	1682
Glu Asn Gly Val Ser Met His Lys Phe Leu Thr Ile Pro Arg Leu Glu	
460 465 470 475	
gaa ctt tac aac act aaa caa ggt cca act aat cca atg ctg ttt cat	1730
Glu Leu Tyr Asn Thr Lys Gln Gly Pro Thr Asn Pro Met Leu Phe His	
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ctt gtt cga gac gtc aaa cag gga aat ctt cct cca gga tat aag atc	1778
Leu Val Arg Asp Val Lys Gln Gly Asn Leu Pro Pro Gly Tyr Lys Ile	
495 500 505	
gct ctg att gat ata gga ctt gtt att gaa tat ctc atg gga gga acc	1826
Ala Leu Ile Asp Ile Gly Leu Val Ile Glu Tyr Leu Met Gly Gly Thr	
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tac aga tgc acc tat act agg aaa cgt ttt cga tta ata tat aat agt	1874
Tyr Arg Cys Thr Tyr Thr Arg Lys Arg Phe Arg Leu Ile Tyr Asn Ser	
525 530 535	
ctt ggt gga aat aat cgg agg tct ggc cga aat acc tcc agc agc act	1922
Leu Gly Gly Asn Asn Arg Arg Ser Gly Arg Asn Thr Ser Ser Ser Thr	
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cct cag ttg cga aag agt cat gaa ttt ttt ggc aat agg gca gat aaa	1970
Pro Gln Leu Arg Lys Ser His Glu Phe Phe Gly Asn Arg Ala Asp Lys	
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Lys Glu Lys Met Arg His Asn His Phe Ile Lys Thr Ala Gln Pro Tyr	
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cga cca aag att gat aca gtt atg gaa gaa gga aag aag aaa aga acc	2066
Arg Pro Lys Ile Asp Thr Val Met Glu Glu Gly Lys Lys Lys Arg Thr	
590 595 600	
aaa gat gaa att gta gac att gat gat cca gaa acc aag cgc ttt cct	2114

Lys	Asp	Glu	Ile	Val	Asp	Ile	Asp	Asp	Pro	Glu	Thr	Lys	Arg	Phe	Pro		
605						610					615						
tat	cca	ctt	aat	gaa	ctt	tta	att	tgg	gct	tgc	ctt	atg	aag	agg	cag	2162	
Tyr	Pro	Leu	Asn	Glu	Leu	Leu	Ile	Trp	Ala	Cys	Leu	Met	Lys	Arg	Gln		
620					625					630					635		
gtc	atg	gcc	cgt	ttt	tta	tgg	caa	cat	ggt	gaa	gaa	tca	atg	gct	aaa	2210	
Val	Met	Ala	Arg	Phe	Leu	Trp	Gln	His	Gly	Glu	Glu	Ser	Met	Ala	Lys		
				640					645					650			
gca	tta	gtt	gcc	tgt	aag	atc	tat	cgt	tca	atg	gca	tat	gaa	gca	aag	2258	
Ala	Leu	Val	Ala	Cys	Lys	Ile	Tyr	Arg	Ser	Met	Ala	Tyr	Glu	Ala	Lys		
			655					660					665				
cag	agt	gac	ctg	gta	gat	gat	act	tca	gaa	gaa	cta	aaa	cag	tat	tcc	2306	
Gln	Ser	Asp	Leu	Val	Asp	Asp	Thr	Ser	Glu	Glu	Leu	Lys	Gln	Tyr	Ser		
		670					675					680					
aat	gat	ttt	ggt	cag	ttg	gcc	gtt	gaa	tta	tta	gaa	cag	tcc	ttc	aga	2354	
Asn	Asp	Phe	Gly	Gln	Leu	Ala	Val	Glu	Leu	Leu	Glu	Gln	Ser	Phe	Arg		
	685					690					695						
caa	gat	gaa	acc	atg	gct	atg	aaa	ttg	ctc	act	tat	gaa	ctg	aag	aac	2402	
Gln	Asp	Glu	Thr	Met	Ala	Met	Lys	Leu	Leu	Thr	Tyr	Glu	Leu	Lys	Asn		
700					705					710					715		
tgg	agt	aat	tca	acc	tgc	ctt	aag	tta	gca	gtt	tct	tca	aga	ctt	aga	2450	
Trp	Ser	Asn	Ser	Thr	Cys	Leu	Lys	Leu	Ala	Val	Ser	Ser	Arg	Leu	Arg		
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cct	ttt	gta	gct	cac	acc	tgt	aca	caa	atg	ttg	tta	tct	gat	atg	tgg	2498	
Pro	Phe	Val	Ala	His	Thr	Cys	Thr	Gln	Met	Leu	Leu	Ser	Asp	Met	Trp		
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atg	gga	agg	ctg	aat	atg	agg	aaa	aat	tcc	tgg	tac	aag	gtc	ata	cta	2546	
Met	Gly	Arg	Leu	Asn	Met	Arg	Lys	Asn	Ser	Trp	Tyr	Lys	Val	Ile	Leu		
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agc	att	tta	gtt	cca	cct	gcc	ata	ttg	ctg	tta	gag	tat	aaa	act	aag	2594	
Ser	Ile	Leu	Val	Pro	Pro	Ala	Ile	Leu	Leu	Leu	Glu	Tyr	Lys	Thr	Lys		
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gct	gaa	atg	tcc	cat	atc	cca	caa	tct	caa	gat	gct	cat	cag	atg	aca	2642	
Ala	Glu	Met	Ser	His	Ile	Pro	Gln	Ser	Gln	Asp	Ala	His	Gln	Met	Thr		
780					785					790					795		
atg	gat	gac	agc	gaa	aac	aac	ttt	cag	aac	ata	aca	gaa	gag	atc	ccc	2690	
Met	Asp	Asp	Ser	Glu	Asn	Asn	Phe	Gln	Asn	Ile	Thr	Glu	Glu	Ile	Pro		
				800					805					810			
atg	gaa	gtg	ttt	aaa	gaa	gta	cgg	att	ttg	gat	agt	aat	gaa	gga	aag	2738	
Met	Glu	Val	Phe	Lys	Glu	Val	Arg	Ile	Leu	Asp	Ser	Asn	Glu	Gly	Lys		
			815					820					825				
aat	gag	atg	gag	ata	caa	atg	aaa	tca	aaa	aag	ctt	cca	att	acg	cga	2786	
Asn	Glu	Met	Glu	Ile	Gln	Met	Lys	Ser	Lys	Lys	Leu	Pro	Ile	Thr	Arg		
		830					835					840					
aag	ttt	tat	gcc	ttt	tat	cat	gca	cca	att	gta	aaa	ttc	tgg	ttt	aac	2834	
Lys	Phe	Tyr	Ala	Phe	Tyr	His	Ala	Pro	Ile	Val	Lys	Phe	Trp	Phe	Asn		

845	850	855	
acg ttg gca tat tta gga ttt ctg atg ctt tat aca ttt gtg gtt ctt			2882
Thr Leu Ala Tyr Leu Gly Phe Leu Met Leu Tyr Thr Phe Val Val Leu			
860	865	870	875
gta caa atg gaa cag tta cct tca gtt caa gaa tgg att gtt att gct			2930
Val Gln Met Glu Gln Leu Pro Ser Val Gln Glu Trp Ile Val Ile Ala			
	880	885	890
tat att ttt act tat gcc att gag aaa gtc cgt gag atc ttt atg tct			2978
Tyr Ile Phe Thr Tyr Ala Ile Glu Lys Val Arg Glu Ile Phe Met Ser			
	895	900	905
gaa gct ggg aaa gta aac cag aag att aaa gta tgg ttt agt gat tac			3026
Glu Ala Gly Lys Val Asn Gln Lys Ile Lys Val Trp Phe Ser Asp Tyr			
	910	915	920
ttc aac atc agt gat aca att gcc ata att tct ttc ttc att gga ttt			3074
Phe Asn Ile Ser Asp Thr Ile Ala Ile Ile Ser Phe Phe Ile Gly Phe			
	925	930	935
gga cta aga ttt gga gca aaa tgg aac ttt gca aat gca tat gat aat			3122
Gly Leu Arg Phe Gly Ala Lys Trp Asn Phe Ala Asn Ala Tyr Asp Asn			
	940	945	950
cat gtt ttt gtg gct gga aga tta att tac tgt ctt aac ata ata ttt			3170
His Val Phe Val Ala Gly Arg Leu Ile Tyr Cys Leu Asn Ile Ile Phe			
	960	965	970
tgg tat gtg cgt ttg cta gat ttt cta gct gta aat caa cag gca gga			3218
Trp Tyr Val Arg Leu Leu Asp Phe Leu Ala Val Asn Gln Gln Ala Gly			
	975	980	985
cct tat gta atg atg att gga aaa atg gtg gcc aat atg ttc tac att			3266
Pro Tyr Val Met Met Ile Gly Lys Met Val Ala Asn Met Phe Tyr Ile			
	990	995	1000
gta gtg att atg gct ctt gta tta ctt agt ttt ggt gtt ccc aga aag			3314
Val Val Ile Met Ala Leu Val Leu Leu Ser Phe Gly Val Pro Arg Lys			
	1005	1010	1015
gca ata ctt tat cct cat gaa gca cca tct tgg act ctt gct aaa gat			3362
Ala Ile Leu Tyr Pro His Glu Ala Pro Ser Trp Thr Leu Ala Lys Asp			
	1020	1025	1030
ata gtt ttt cac cca tac tgg atg att ttt ggt gaa gtt tat gca tac			3410
Ile Val Phe His Pro Tyr Trp Met Ile Phe Gly Glu Val Tyr Ala Tyr			
	1040	1045	1050
gaa att gat gtg tgt gca aat gat tct gtt atc cct caa atc tgt ggt			3458
Glu Ile Asp Val Cys Ala Asn Asp Ser Val Ile Pro Gln Ile Cys Gly			
	1055	1060	1065
cct ggg acg tgg ttg act cca ttt ctt caa gca gtc tac ctc ttt gta			3506
Pro Gly Thr Trp Leu Thr Pro Phe Leu Gln Ala Val Tyr Leu Phe Val			
	1070	1075	1080
cag tat atc att atg gtt aat ctt ctt att gca ttt ttc aac aat gtg			3554
Gln Tyr Ile Ile Met Val Asn Leu Leu Ile Ala Phe Phe Asn Asn Val			
	1085	1090	1095

tat tta caa gtg aag gca att tcc aat att gta tgg aag tac cag cgt	3602
Tyr Leu Gln Val Lys Ala Ile Ser Asn Ile Val Trp Lys Tyr Gln Arg	
1100 1105 1110 1115	
tat cat ttt att atg gct tat cat gag aaa cca gtt ctg cct cct cca	3650
Tyr His Phe Ile Met Ala Tyr His Glu Lys Pro Val Leu Pro Pro Pro	
1120 1125 1130	
ctt atc att ctt agc cat ata gtt tct ctg ttt tgc tgc ata tgt aag	3698
Leu Ile Ile Leu Ser His Ile Val Ser Leu Phe Cys Cys Ile Cys Lys	
1135 1140 1145	
aga aga aag aaa gat aag act tcc gat gga cca aaa ctt ttc tta aca	3746
Arg Arg Lys Lys Asp Lys Thr Ser Asp Gly Pro Lys Leu Phe Leu Thr	
1150 1155 1160	
gaa gaa gat caa aag aaa ctt cat gat ttt gaa gag cag tgt gtt gaa	3794
Glu Glu Asp Gln Lys Lys Leu His Asp Phe Glu Glu Gln Cys Val Glu	
1165 1170 1175	
atg tat ttc aat gaa aaa gat gac aaa ttt cat tct ggg agt gaa gag	3842
Met Tyr Phe Asn Glu Lys Asp Asp Lys Phe His Ser Gly Ser Glu Glu	
1180 1185 1190 1195	
aga att cgt gtc act ttt gaa aga gtg gaa cag atg tgc att cag att	3890
Arg Ile Arg Val Thr Phe Glu Arg Val Glu Gln Met Cys Ile Gln Ile	
1200 1205 1210	
aaa gaa gtt gga gat cgt gtc aac tac ata aaa aga tca tta caa tca	3938
Lys Glu Val Gly Asp Arg Val Asn Tyr Ile Lys Arg Ser Leu Gln Ser	
1215 1220 1225	
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Leu Asp Ser Gln Ile Gly His Leu Gln Asp Leu Ser Ala Leu Thr Val	
1230 1235 1240	
gat aca tta aaa aca ctc act gcc cag aaa gcg tcg gaa gct agc aaa	4034
Asp Thr Leu Lys Thr Leu Thr Ala Gln Lys Ala Ser Glu Ala Ser Lys	
1245 1250 1255	
gtt cat aat gaa atc aca cga gaa ctg agc att tcc aaa cac ttg gct	4082
Val His Asn Glu Ile Thr Arg Glu Leu Ser Ile Ser Lys His Leu Ala	
1260 1265 1270 1275	
caa aac ctt att gat gat ggt cct gta aga cct tct gta tgg aaa aag	4130
Gln Asn Leu Ile Asp Asp Gly Pro Val Arg Pro Ser Val Trp Lys Lys	
1280 1285 1290	
cat ggt gtt gta aat aca ctt agc tcc tct ctt cct caa ggt gat ctt	4178
His Gly Val Val Asn Thr Leu Ser Ser Ser Leu Pro Gln Gly Asp Leu	
1295 1300 1305	
gaa agt aat aat cct ttt cat tgt aat att tta atg aaa gat gac aaa	4226
Glu Ser Asn Asn Pro Phe His Cys Asn Ile Leu Met Lys Asp Asp Lys	
1310 1315 1320	
gat ccc cag tgt aat ata ttt ggt caa gac tta cct gca gta ccc cag	4274
Asp Pro Gln Cys Asn Ile Phe Gly Gln Asp Leu Pro Ala Val Pro Gln	
1325 1330 1335	

aga aaa gaa ttt aat ttt cca gag gct ggt tcc tct tct ggt gcc tta	4322
Arg Lys Glu Phe Asn Phe Pro Glu Ala Gly Ser Ser Ser Gly Ala Leu	
1340 1345 1350 1355	
ttc cca agt gct gtt tcc cct cca gaa ctg cga cag aga cta cat ggg	4370
Phe Pro Ser Ala Val Ser Pro Pro Glu Leu Arg Gln Arg Leu His Gly	
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Val Glu Leu Leu Lys Ile Phe Asn Lys Asn Gln Lys Leu Gly Ser Ser	
1375 1380 1385	
tct act agc ata cca cat ctg tca tcc cca cca acc aaa ttt ttt gtt	4466
Ser Thr Ser Ile Pro His Leu Ser Ser Pro Pro Thr Lys Phe Phe Val	
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agt aca cca tct cag cca agt tgc aaa agc cac ttg gaa act gga acc	4514
Ser Thr Pro Ser Gln Pro Ser Cys Lys Ser His Leu Glu Thr Gly Thr	
1405 1410 1415	
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Lys Asp Gln Glu Thr Val Cys Ser Lys Ala Thr Glu Gly Asp Asn Thr	
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gaa ttt gga gca ttt gta gga cac aga gat agc atg gat tta cag agg	4610
Glu Phe Gly Ala Phe Val Gly His Arg Asp Ser Met Asp Leu Gln Arg	
1440 1445 1450	
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Phe Lys Glu Thr Ser Asn Lys Ile Lys Ile Leu Ser Glu Gln Gln Asn	
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gat gta aga aat gtg att atg gag tat aca gag atg cct aaa tat gag	4706
Asp Val Arg Asn Val Ile Met Glu Tyr Thr Glu Met Pro Lys Tyr Glu	
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aat aac aat act tct gaa aac act ttg aaa cga gtg agt tct ctt gct	4754
Asn Asn Asn Thr Ser Glu Asn Thr Leu Lys Arg Val Ser Ser Leu Ala	
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Gly Phe Thr Asp Cys His Arg Thr Ser Ile Pro Val His Ser Lys Gln	
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Ala Glu Lys Ile Ser Arg Arg Pro Ser Thr Glu Asp Thr His Glu Val	
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Asp Ser Lys Ala Ala Leu Ile Pro Asp Trp Leu Gln Asp Arg Pro Ser	
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Asn Arg Glu Met Pro Ser Glu Glu Gly Thr Leu Asn Gly Leu Thr Ser	
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Pro Phe Lys Pro Ala Met Asp Thr Asn Tyr Tyr Tyr Ser Ala Val Glu	
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aga aat aac ttg atg agg tta tca cag agc att cca ttt aca cct gtg	5042

Arg Asn Asn Leu Met	Arg Leu Ser Gln Ser Ile Pro Phe Thr Pro Val	
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Pro Pro Arg Gly Glu Pro Val Thr Val Tyr Arg Leu Glu Glu Ser Ser		
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ccc aac ata cta aat aac agc atg tct tct tgg tca caa cta ggc ctc		5138
Pro Asn Ile Leu Asn Asn Ser Met Ser Ser Trp Ser Gln Leu Gly Leu		
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tgt gcc aaa ata gag ttt tta agc aaa gag gag atg gga gga ggt tta		5186
Cys Ala Lys Ile Glu Phe Leu Ser Lys Glu Glu Met Gly Gly Gly Leu		
1630	1635	1640
cga aga gct gtc aaa gta cag tgt acc tgg tca gaa cat gat atc ctc		5234
Arg Arg Ala Val Lys Val Gln Cys Thr Trp Ser Glu His Asp Ile Leu		
1645	1650	1655
aaa tca ggg cat ctt tat att atc aaa tct ttt ctt cca gag gtg gtt		5282
Lys Ser Gly His Leu Tyr Ile Ile Lys Ser Phe Leu Pro Glu Val Val		
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Asn Thr Trp Ser Ser Ile Tyr Lys Glu Asp Thr Val Leu His Leu Cys		
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ctg aga gaa att caa caa cag aga gca gca caa aag ctt acg ttt gcc		5378
Leu Arg Glu Ile Gln Gln Gln Arg Ala Ala Gln Lys Leu Thr Phe Ala		
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Phe Asn Gln Met Lys Pro Lys Ser Ile Pro Tyr Ser Pro Arg Phe Leu		
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Glu Val Phe Leu Leu Tyr Cys His Ser Ala Gly Gln Trp Phe Ala Val		
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Glu Glu Cys Met Thr Gly Glu Phe Arg Lys Tyr Asn Asn Asn Asn Gly		
1740	1745	1750 1755
gat gag att att cca act aat act ctg gaa gag atc atg cta gcc ttt		5570
Asp Glu Ile Ile Pro Thr Asn Thr Leu Glu Glu Ile Met Leu Ala Phe		
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agc cac tgg act tac gaa tat aca aga ggg gag tta ctg gta ctt gat		5618
Ser His Trp Thr Tyr Glu Tyr Thr Arg Gly Glu Leu Leu Val Leu Asp		
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Leu Gln Gly Val Gly Glu Asn Leu Thr Asp Pro Ser Val Ile Lys Ala		
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Glu Glu Lys Arg Ser Cys Asp Met Val Phe Gly Pro Ala Asn Leu Gly		
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Glu Asp Ala Ile Lys Asn Phe Arg Ala Lys His His Cys Asn Ser Cys		

1820 1825 1830 1835

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 1840 1845 1850

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 Asp Lys Ile Ile Phe Pro Gln Asp Glu Pro Ser Asp Leu Asn Leu Gln
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 Pro Gly Asn Ser Thr Lys Glu Ser Glu Ser Thr Asn Ser Val Arg Leu
 1870 1875 1880

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 Met Leu
 1885

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<211> 1885

<212> PRT

<213> Homo sapiens

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 Val Tyr Ile Ile Pro Ser Ser Lys Asp Pro His Arg Cys Leu Pro Gly
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 35 40 45
 Lys Gln His Ala Cys Phe Thr Ala Ser Leu Ala Met Lys Tyr Ser Asp
 50 55 60
 Val Lys Leu Gly Asp His Phe Asn Gln Ala Ile Glu Glu Trp Ser Val
 65 70 75 80
 Glu Lys His Thr Glu Gln Ser Pro Thr Asp Ala Tyr Gly Val Ile Asn
 85 90 95
 Phe Gln Gly Gly Ser His Ser Tyr Arg Ala Lys Tyr Val Arg Leu Ser

10.

Thr Val Met Glu Glu Gly Lys Lys Lys Arg Thr Lys Asp Glu Ile Val
 595 600 605
 Asp Ile Asp Asp Pro Glu Thr Lys Arg Phe Pro Tyr Pro Leu Asn Glu
 610 615 620
 Leu Leu Ile Trp Ala Cys Leu Met Lys Arg Gln Val Met Ala Arg Phe
 625 630 635 640
 Leu Trp Gln His Gly Glu Glu Ser Met Ala Lys Ala Leu Val Ala Cys
 645 650 655
 Lys Ile Tyr Arg Ser Met Ala Tyr Glu Ala Lys Gln Ser Asp Leu Val
 660 665 670
 Asp Asp Thr Ser Glu Glu Leu Lys Gln Tyr Ser Asn Asp Phe Gly Gln
 675 680 685
 Leu Ala Val Glu Leu Leu Glu Gln Ser Phe Arg Gln Asp Glu Thr Met
 690 695 700
 Ala Met Lys Leu Leu Thr Tyr Glu Leu Lys Asn Trp Ser Asn Ser Thr
 705 710 715 720
 Cys Leu Lys Leu Ala Val Ser Ser Arg Leu Arg Pro Phe Val Ala His
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 Thr Cys Thr Gln Met Leu Leu Ser Asp Met Trp Met Gly Arg Leu Asn
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 Met Arg Lys Asn Ser Trp Tyr Lys Val Ile Leu Ser Ile Leu Val Pro
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 Pro Ala Ile Leu Leu Leu Glu Tyr Lys Thr Lys Ala Glu Met Ser His
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 Ile Pro Gln Ser Gln Asp Ala His Gln Met Thr Met Asp Asp Ser Glu
 785 790 795 800
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 Gln Met Lys Ser Lys Lys Leu Pro Ile Thr Arg Lys Phe Tyr Ala Phe
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 Tyr His Ala Pro Ile Val Lys Phe Trp Phe Asn Thr Leu Ala Tyr Leu
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 Gly Phe Leu Met Leu Tyr Thr Phe Val Val Leu Val Gln Met Glu Gln
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 Leu Pro Ser Val Gln Glu Trp Ile Val Ile Ala Tyr Ile Phe Thr Tyr
 885 890 895
 Ala Ile Glu Lys Val Arg Glu Ile Phe Met Ser Glu Ala Gly Lys Val
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 Ala Lys Trp Asn Phe Ala Asn Ala Tyr Asp Asn His Val Phe Val Ala
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 Gly Arg Leu Ile Tyr Cys Leu Asn Ile Ile Phe Trp Tyr Val Arg Leu
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Val Asn Leu Leu Ile Ala Phe Phe Asn Asn Val Tyr Leu Gln Val Lys		
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Ala Ile Ser Asn Ile Val Trp Lys Tyr Gln Arg Tyr His Phe Ile Met		
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Ala Tyr His Glu Lys Pro Val Leu Pro Pro Pro Leu Ile Ile Leu Ser		
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His Ile Val Ser Leu Phe Cys Cys Ile Cys Lys Arg Arg Lys Lys Asp		
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Lys Thr Ser Asp Gly Pro Lys Leu Phe Leu Thr Glu Glu Asp Gln Lys		
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Lys Leu His Asp Phe Glu Glu Gln Cys Val Glu Met Tyr Phe Asn Glu		
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Lys Asp Asp Lys Phe His Ser Gly Ser Glu Glu Arg Ile Arg Val Thr		
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Phe Glu Arg Val Glu Gln Met Cys Ile Gln Ile Lys Glu Val Gly Asp		
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Arg Val Asn Tyr Ile Lys Arg Ser Leu Gln Ser Leu Asp Ser Gln Ile		
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Gly His Leu Gln Asp Leu Ser Ala Leu Thr Val Asp Thr Leu Lys Thr		
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Leu Thr Ala Gln Lys Ala Ser Glu Ala Ser Lys Val His Asn Glu Ile		
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Thr Arg Glu Leu Ser Ile Ser Lys His Leu Ala Gln Asn Leu Ile Asp		
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Asp Gly Pro Val Arg Pro Ser Val Trp Lys Lys His Gly Val Val Asn		
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Thr Leu Ser Ser Ser Leu Pro Gln Gly Asp Leu Glu Ser Asn Asn Pro		
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Phe His Cys Asn Ile Leu Met Lys Asp Asp Lys Asp Pro Gln Cys Asn		
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Phe Pro Glu Ala Gly Ser Ser Ser Gly Ala Leu Phe Pro Ser Ala Val		
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Ser Pro Pro Glu Leu Arg Gln Arg Leu His Gly Val Glu Leu Leu Lys		
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Ile Phe Asn Lys Asn Gln Lys Leu Gly Ser Ser Ser Thr Ser Ile Pro		
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His Leu Ser Ser Pro Pro Thr Lys Phe Phe Val Ser Thr Pro Ser Gln		
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Val Cys Ser Lys Ala Thr Glu Gly Asp Asn Thr Glu Phe Gly Ala Phe		
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Val Gly His Arg Asp Ser Met Asp Leu Gln Arg Phe Lys Glu Thr Ser		
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Asn Lys Ile Lys Ile Leu Ser Glu Gln Gln Asn Asp Val Arg Asn Val		
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Leu Ile Pro Asp Trp Leu Gln Asp Arg Pro Ser Asn Arg Glu Met Pro		
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Ser Glu Glu Gly Thr Leu Asn Gly Leu Thr Ser Pro Phe Lys Pro Ala		
1555	1560	1565


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Arg Leu Ser Gln Ser Ile Pro Phe Thr Pro Val Pro Pro Arg Gly Glu
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Pro Val Thr Val Tyr Arg Leu Glu Glu Ser Ser Pro Asn Ile Leu Asn
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Asn Ser Met Ser Ser Trp Ser Gln Leu Gly Leu Cys Ala Lys Ile Glu
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Phe Leu Ser Lys Glu Glu Met Gly Gly Gly Leu Arg Arg Ala Val Lys
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Val Gln Cys Thr Trp Ser Glu His Asp Ile Leu Lys Ser Gly His Leu
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Tyr Ile Ile Lys Ser Phe Leu Pro Glu Val Val Asn Thr Trp Ser Ser
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Ile Tyr Lys Glu Asp Thr Val Leu His Leu Cys Leu Arg Glu Ile Gln
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Gln Gln Arg Ala Ala Gln Lys Leu Thr Phe Ala Phe Asn Gln Met Lys
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Pro Lys Ser Ile Pro Tyr Ser Pro Arg Phe Leu Glu Val Phe Leu Leu
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Tyr Cys His Ser Ala Gly Gln Trp Phe Ala Val Glu Glu Cys Met Thr
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Gly Glu Phe Arg Lys Tyr Asn Asn Asn Asn Gly Asp Glu Ile Ile Pro
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Thr Asn Thr Leu Glu Glu Ile Met Leu Ala Phe Ser His Trp Thr Tyr
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Glu Tyr Thr Arg Gly Glu Leu Leu Val Leu Asp Leu Gln Gly Val Gly
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Glu Asn Leu Thr Asp Pro Ser Val Ile Lys Ala Glu Glu Lys Arg Ser
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Cys Asp Met Val Phe Gly Pro Ala Asn Leu Gly Glu Asp Ala Ile Lys
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Asn Phe Arg Ala Lys His His Cys Asn Ser Cys Cys Arg Lys Leu Lys
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Pro Gln Asp Glu Pro Ser Asp Leu Asn Leu Gln Pro Gly Asn Ser Thr
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Val Tyr Ile Ile Pro Ser Ser Lys Asp Pro His Arg Cys Leu Pro Gly
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Lys	Gln	His	Ala	Cys	Phe	Thr	Ala	Ser	Leu	Ala	Met	Lys	Tyr	Ser	Asp	
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Phe	Gln	Gly	Gly	Ser	His	Ser	Tyr	Arg	Ala	Lys	Tyr	Val	Arg	Leu	Ser	
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Tyr	Asp	Thr	Lys	Pro	Glu	Val	Ile	Leu	Gln	Leu	Leu	Leu	Lys	Glu	Trp	
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Gln	Met	Glu	Leu	Pro	Lys	Leu	Val	Ile	Ser	Val	His	Gly	Gly	Met	Gln	
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Lys	Phe	Glu	Leu	His	Pro	Arg	Ile	Lys	Gln	Leu	Leu	Gly	Lys	Gly	Leu	
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Ser	Arg	Ser	Ser	Arg	Lys	Ile	Cys	Thr	Ile	Gly	Ile	Ala	Pro	Trp	Gly	
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Tyr	Gln	Thr	Leu	Leu	Asn	Pro	Leu	Ser	Lys	Leu	Asn	Val	Leu	Asn	Asn	
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Gln	Arg	Ile	His	Ala	Arg	Ile	Gly	Gln	Gly	Val	Pro	Val	Val	Ala	Leu	

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Gln Glu Ser Pro Pro Val Pro Val Val Val Cys Glu Gly Thr Gly Arg			
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Ala Ala Asp Leu Leu Ala Tyr Ile His Lys Gln Thr Glu Glu Gly Gly			
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Asn Leu Pro Asp Ala Ala Gly His Asp Ile Ile Ser Thr Ile Lys Lys			
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Met Glu Cys Met Lys Arg Lys Glu Leu Ile Thr Val Phe His Ile Gly			
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Lys Gly Thr Asn Ala Ser Ala Phe Asp Gln Leu Ile Leu Thr Leu Ala			
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Trp Gly Arg Val Asp Ile Ala Lys Asn His Val Phe Val Tyr Gly Gln			
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Gln Trp Leu Val Gly Ser Leu Glu Gln Ala Met Leu Asp Ala Leu Val			
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Met Asp Arg Val Ala Phe Val Lys Leu Leu Ile Glu Asn Gly Val Ser			
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Met His Lys Phe Leu Thr Ile Pro Arg Leu Glu Glu Leu Tyr Asn Thr			
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Lys Gln Gly Pro Thr Asn Pro Met Leu Phe His Leu Val Arg Asp Val			
485	490	495	
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Gly Leu Val Ile Glu Tyr Leu Met Gly Gly Thr Tyr Arg Cys Thr Tyr			
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Thr	Arg	Lys	Arg	Phe	Arg	Leu	Ile	Tyr	Asn	Ser	Leu	Gly	Gly	Asn	Asn	
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		1155				1160							1165				
aaa	ctt	cat	gat	ttt	gaa	gag	cag	tgt	gtt	gaa	atg	tat	ttc	aat	gaa	3552	
Lys	Leu	His	Asp	Phe	Glu	Glu	Gln	Cys	Val	Glu	Met	Tyr	Phe	Asn	Glu		
	1170					1175				1180							
aaa	gat	gac	aaa	ttt	cat	tct	ggg	agt	gaa	gag	aga	att	cgt	gtc	act	3600	
Lys	Asp	Asp	Lys	Phe	His	Ser	Gly	Ser	Glu	Glu	Arg	Ile	Arg	Val	Thr		
1185					1190				1195						1200		
ttt	gaa	aga	gtg	gaa	cag	atg	tgc	att	cag	att	aaa	gaa	gtt	gga	gat	3648	
Phe	Glu	Arg	Val	Glu	Gln	Met	Cys	Ile	Gln	Ile	Lys	Glu	Val	Gly	Asp		
				1205					1210						1215		
cgt	gtc	aac	tac	ata	aaa	aga	tca	tta	caa	tca	tta	gat	tct	caa	att	3696	
Arg	Val	Asn	Tyr	Ile	Lys	Arg	Ser	Leu	Gln	Ser	Leu	Asp	Ser	Gln	Ile		
			1220					1225							1230		
ggc	cat	ttg	caa	gat	ctt	tca	gcc	ctg	acg	gta	gat	aca	tta	aaa	aca	3744	
Gly	His	Leu	Gln	Asp	Leu	Ser	Ala	Leu	Thr	Val	Asp	Thr	Leu	Lys	Thr		
		1235					1240						1245				
ctc	act	gcc	cag	aaa	gcg	tcg	gaa	gct	agc	aaa	gtt	cat	aat	gaa	atc	3792	
Leu	Thr	Ala	Gln	Lys	Ala	Ser	Glu	Ala	Ser	Lys	Val	His	Asn	Glu	Ile		

1250	1255	1260	
aca cga gaa ctg agc att tcc aaa cac ttg gct caa aac ctt att gat			3840
Thr Arg Glu Leu Ser Ile Ser Lys His Leu Ala Gln Asn Leu Ile Asp			
1265	1270	1275	1280
gat ggt cct gta aga cct tct gta tgg aaa aag cat ggt gtt gta aat			3888
Asp Gly Pro Val Arg Pro Ser Val Trp Lys Lys His Gly Val Val Asn			
1285	1290	1295	
aca ctt agc tcc tct ctt cct caa ggt gat ctt gaa agt aat aat cct			3936
Thr Leu Ser Ser Ser Leu Pro Gln Gly Asp Leu Glu Ser Asn Asn Pro			
1300	1305	1310	
ttt cat tgt aat att tta atg aaa gat gac aaa gat ccc cag tgt aat			3984
Phe His Cys Asn Ile Leu Met Lys Asp Asp Lys Asp Pro Gln Cys Asn			
1315	1320	1325	
ata ttt ggt caa gac tta cct gca gta ccc cag aga aaa gaa ttt aat			4032
Ile Phe Gly Gln Asp Leu Pro Ala Val Pro Gln Arg Lys Glu Phe Asn			
1330	1335	1340	
ttt cca gag gct ggt tcc tct tct ggt gcc tta ttc cca agt gct gtt			4080
Phe Pro Glu Ala Gly Ser Ser Ser Gly Ala Leu Phe Pro Ser Ala Val			
1345	1350	1355	1360
tcc cct cca gaa ctg cga cag aga cta cat ggg gta gaa ctc tta aaa			4128
Ser Pro Pro Glu Leu Arg Gln Arg Leu His Gly Val Glu Leu Leu Lys			
1365	1370	1375	
ata ttt aat aaa aat caa aaa tta ggc agt tca tct act agc ata cca			4176
Ile Phe Asn Lys Asn Gln Lys Leu Gly Ser Ser Ser Thr Ser Ile Pro			
1380	1385	1390	
cat ctg tca tcc cca cca acc aaa ttt ttt gtt agt aca cca tct cag			4224
His Leu Ser Ser Pro Pro Thr Lys Phe Phe Val Ser Thr Pro Ser Gln			
1395	1400	1405	
cca agt tgc aaa agc cac ttg gaa act gga acc aaa gat caa gaa act			4272
Pro Ser Cys Lys Ser His Leu Glu Thr Gly Thr Lys Asp Gln Glu Thr			
1410	1415	1420	
gtt tgc tct aaa gct aca gaa gga gat aat aca gaa ttt gga gca ttt			4320
Val Cys Ser Lys Ala Thr Glu Gly Asp Asn Thr Glu Phe Gly Ala Phe			
1425	1430	1435	1440
gta gga cac aga gat agc atg gat tta cag agg ttt aaa gaa aca tca			4368
Val Gly His Arg Asp Ser Met Asp Leu Gln Arg Phe Lys Glu Thr Ser			
1445	1450	1455	
aac aag ata aaa ata cta tcc gag cag cag aat gat gta aga aat gtg			4416
Asn Lys Ile Lys Ile Leu Ser Glu Gln Gln Asn Asp Val Arg Asn Val			
1460	1465	1470	
att atg gag tat aca gag atg cct aaa tat gag aat aac aat act tct			4464
Ile Met Glu Tyr Thr Glu Met Pro Lys Tyr Glu Asn Asn Asn Thr Ser			
1475	1480	1485	
gaa aac act ttg aaa cga gtg agt tct ctt gct gga ttt act gac tgt			4512
Glu Asn Thr Leu Lys Arg Val Ser Ser Leu Ala Gly Phe Thr Asp Cys			
1490	1495	1500	

cac aga act tcc att cct gtt cat tca aaa caa gca gaa aaa atc agt	4560
His Arg Thr Ser Ile Pro Val His Ser Lys Gln Ala Glu Lys Ile Ser	
1505 1510 1515 1520	
aga agg cca tct acc gaa gac act cat gaa gta gat tcc aaa gca gct	4608
Arg Arg Pro Ser Thr Glu Asp Thr His Glu Val Asp Ser Lys Ala Ala	
1525 1530 1535	
tta ata ccg gat tgg tta caa gat aga cca tca aac aga gaa atg cca	4656
Leu Ile Pro Asp Trp Leu Gln Asp Arg Pro Ser Asn Arg Glu Met Pro	
1540 1545 1550	
tct gaa gaa gga aca tta aat ggt ctc act tct cca ttt aag cca gct	4704
Ser Glu Glu Gly Thr Leu Asn Gly Leu Thr Ser Pro Phe Lys Pro Ala	
1555 1560 1565	
atg gat aca aat tac tat tat tca gct gtg gaa aga aat aac ttg atg	4752
Met Asp Thr Asn Tyr Tyr Tyr Ser Ala Val Glu Arg Asn Asn Leu Met	
1570 1575 1580	
agg tta tca cag agc att cca ttt aca cct gtg cct cca aga ggg gag	4800
Arg Leu Ser Gln Ser Ile Pro Phe Thr Pro Val Pro Pro Arg Gly Glu	
1585 1590 1595 1600	
cct gtc aca gtg tat cgt ttg gaa gag agt tca ccc aac ata cta aat	4848
Pro Val Thr Val Tyr Arg Leu Glu Glu Ser Ser Pro Asn Ile Leu Asn	
1605 1610 1615	
aac agc atg tct tct tgg tca caa cta ggc ctc tgt gcc aaa ata gag	4896
Asn Ser Met Ser Ser Trp Ser Gln Leu Gly Leu Cys Ala Lys Ile Glu	
1620 1625 1630	
ttt tta agc aaa gag gag atg gga gga ggt tta cga aga gct gtc aaa	4944
Phe Leu Ser Lys Glu Glu Met Gly Gly Gly Leu Arg Arg Ala Val Lys	
1635 1640 1645	
gta cag tgt acc tgg tca gaa cat gat atc ctc aaa tca ggg cat ctt	4992
Val Gln Cys Thr Trp Ser Glu His Asp Ile Leu Lys Ser Gly His Leu	
1650 1655 1660	
tat att atc aaa tct ttt ctt cca gag gtg gtt aat aca tgg tca agt	5040
Tyr Ile Ile Lys Ser Phe Leu Pro Glu Val Val Asn Thr Trp Ser Ser	
1665 1670 1675 1680	
att tat aaa gaa gat aca gtt ctg cat ctc tgt ctg aga gaa att caa	5088
Ile Tyr Lys Glu Asp Thr Val Leu His Leu Cys Leu Arg Glu Ile Gln	
1685 1690 1695	
caa cag aga gca gca caa aag ctt acg ttt gcc ttt aat caa atg aaa	5136
Gln Gln Arg Ala Ala Gln Lys Leu Thr Phe Ala Phe Asn Gln Met Lys	
1700 1705 1710	
ccc aaa tcc ata cca tat tct cca agg ttc ctt gaa gtt ttc ctg ctg	5184
Pro Lys Ser Ile Pro Tyr Ser Pro Arg Phe Leu Glu Val Phe Leu Leu	
1715 1720 1725	
tat tgc cat tca gca gga cag tgg ttt gct gtg gaa gaa tgt atg act	5232
Tyr Cys His Ser Ala Gly Gln Trp Phe Ala Val Glu Glu Cys Met Thr	
1730 1735 1740	

gga gaa ttt aga aaa tac aac aat aat aat gga gat gag att att cca	5280
Gly Glu Phe Arg Lys Tyr Asn Asn Asn Asn Gly Asp Glu Ile Ile Pro	
1745 1750 1755 1760	
act aat act ctg gaa gag atc atg cta gcc ttt agc cac tgg act tac	5328
Thr Asn Thr Leu Glu Glu Ile Met Leu Ala Phe Ser His Trp Thr Tyr	
1765 1770 1775	
gaa tat aca aga ggg gag tta ctg gta ctt gat ttg caa ggt gtt ggt	5376
Glu Tyr Thr Arg Gly Glu Leu Leu Val Leu Asp Leu Gln Gly Val Gly	
1780 1785 1790	
gaa aat ttg act gac cca tct gtg ata aaa gca gaa gaa aag aga tcc	5424
Glu Asn Leu Thr Asp Pro Ser Val Ile Lys Ala Glu Glu Lys Arg Ser	
1795 1800 1805	
tgt gat atg gtt ttt ggc cca gca aat cta gga gaa gat gca att aaa	5472
Cys Asp Met Val Phe Gly Pro Ala Asn Leu Gly Glu Asp Ala Ile Lys	
1810 1815 1820	
aac ttc aga gca aaa cat cac tgt aat tct tgc tgt aga aag ctt aaa	5520
Asn Phe Arg Ala Lys His His Cys Asn Ser Cys Cys Arg Lys Leu Lys	
1825 1830 1835 1840	
ctt cca gat ctg aag agg aat gat tat acg cct gat aaa att ata ttt	5568
Leu Pro Asp Leu Lys Arg Asn Asp Tyr Thr Pro Asp Lys Ile Ile Phe	
1845 1850 1855	
cct cag gat gag cct tca gat ttg aat ctt cag cct gga aat tcc acc	5616
Pro Gln Asp Glu Pro Ser Asp Leu Asn Leu Gln Pro Gly Asn Ser Thr	
1860 1865 1870	
aaa gaa tca gaa tca act aat tct gtt cgt ctg atg tta	5655
Lys Glu Ser Glu Ser Thr Asn Ser Val Arg Leu Met Leu	
1875 1880 1885	

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 February 2002 (07.02.2002)

PCT

(10) International Publication Number
WO 02/010391 A3

(51) International Patent Classification⁷: **C12N 15/12**,
C07K 14/705, 16/28, C12Q 1/68, G01N 33/68

(21) International Application Number: PCT/US01/24190

(22) International Filing Date: 31 July 2001 (31.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/221,925 31 July 2000 (31.07.2000) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

Published:

— with international search report

(88) Date of publication of the international search report:
27 December 2002

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.



WO 02/010391 A3

(54) Title: 18610, A HUMAN TRANSIENT RECEPTOR AND USES THEREOF

(57) Abstract: The invention provides isolated nucleic acids molecules, designated TR-1 nucleic acid molecules, which encode novel transient receptor potential channel molecules. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing TR-1 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a TR-1 gene has been introduced or disrupted. The invention still further provides isolated TR-1 polypeptides, fusion polypeptides, antigenic peptides and anti-TR-1 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/24190

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/705 C07K16/28 C12Q1/68 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CAB Data, SEQUENCE SEARCH, BIOSIS, EPO-Internal, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RYAZANOV A G ET AL: "Alpha-kinases: a new class of protein kinases with a novel catalytic domain." CURRENT BIOLOGY: CB. ENGLAND 28 JAN 1999, vol. 9, no. 2, 28 January 1999 (1999-01-28), pages R43-R45, XP002209314 ISSN: 0960-9822 Melanoma K, position 58 to 279 is identical to SEQ ID No. 2, position 1624 to 1844; figure 1	1-16
X	--- WO 00 40614 A (BETH ISRAEL HOSPITAL ;SCHARENBERG ANDREW M (US)) 13 July 2000 (2000-07-13) SEQ ID NOS. 27 and 28; claims 1-36 --- -/--	1-26

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

9 August 2002

Date of mailing of the international search report

24.08.2002

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/24190

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL SEQUENCE DATABASE [Online] EMBL-EBI, Hinxton, UK; 2 June 2000 (2000-06-02) M. MATSUSHITA: "Mus musculus transient receptor potential-related protein (Chak) mRNA" Database accession no. AF149013 XP002209316 abstract</p>	5-16
X	<p>--- DATABASE EMBL ASEQUENCE DATABASE [Online] EMBL-EBI, Hinxton, UK; 22 February 2000 (2000-02-22) S. SUGANO ET AL.: "Homo sapiens cDNA FLJ20117 fis, clone COL05727" Database accession no. AK000124 XP002209317 abstract</p>	5-16
L	<p>--- DATABASE GENESEQ SEQUENCE DATABASE [Online] NCBI, Washington, US; 6 November 2001 (2001-11-06) T. OTA ET AL.: "Human full length cDNA, SEQ ID NO: 3419" Database accession no. AAK94537 XP002209318 for further information abstract</p>	
E	<p>-& EP 1 130 094 A (HELIX RESEARCH INSTITUTE) 5 September 2001 (2001-09-05) SEQ ID NO. 3419; claims 1-23</p>	1-26
E	<p>--- WO 02 12340 A (INCYTE GENOMICS INC) 14 February 2002 (2002-02-14) SEQ ID NOs. 23 and 53; claims 1-44,67</p>	1-26
L	<p>--- DATABASE EMBL SEQUENCE DATABASE [Online] EMBL-EBI, Hinxton, UK; 16 July 2002 (2002-07-16) B.D. ZERHUSEN ET AL.: "Sequence 5 from W00214368" Database accession no. AX465477 XP002209319 for further information abstract</p>	
E	<p>-& WO 02 14368 A (CURAGEN CORPORATION) 21 February 2002 (2002-02-21) Sequence ID No. 5; claims 1-49</p>	1-26
	<p>--- -/--</p>	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/24190

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
L P,X	<p>DATABASE EMBL SEQUENCE DATABASE [Online] EMBL-EBI, Hinxton, UK; 9 May 2001 (2001-05-09) M.J. NADLER ET AL.: "Homo sapiens LTRPC7 mRNA, complete cds." Database accession no. AY032950 XP002209320 abstract -& M.J.S. NADLER ET AL.: "LTRPC7 is a Mg-ATP-regulated divalent cation channel required for cell viability" NATURE, vol. 411, 31 May 2001 (2001-05-31), pages 590-595, XP002209315 MACMILLAN JOURNALS LTD., LONDON,UK the whole document -----</p>	1-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/24190

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 25-partially (as far as in vivo methods are concerned) is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 25-partially
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 25-partially

Present claim 25 relate to a method of modulating the activity of the 18610-polypeptide or an 16836-expressing cell by using an agent which binds to said 18610-polypeptide without giving a true technical characterization. Moreover no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to antibodies, antisense molecules, ribozymes, triple helix molecules, polypeptides and nucleic acids, the structure of which can be directly derived from SEQ ID Nos. 1-3.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/24190

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0040614	A	13-07-2000	AU 2055600 A EP 1141017 A2 WO 0040614 A2	24-07-2000 10-10-2001 13-07-2000
EP 1130094	A	05-09-2001	EP 1130094 A2 JP 2002017375 A	05-09-2001 22-01-2002
WO 0212340	A		NONE	
WO 0214368	A	21-02-2002	AU 8496601 A WO 0214368 A2 AU 4982801 A WO 0174897 A2	25-02-2002 21-02-2002 15-10-2001 11-10-2001

